SPECIAL EDITION

SCIENTIFIC AMERICAN

New Answers for CANCER

Cutting-edge drugs and research are helping solve the puzzle

Cancer Resource Guide

Where to turn for help

Stem Cells

The real culprits?

The Cancer Genome

Mapping out an attack plan

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SCIENTIFIC AMERICAN

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letter from the editor

In This Issue

KENN BROWN *Mondolithic Studios*

NEARLY 40 YEARS since we declared war on cancer, how goes the campaign against this intractable and ancient adversary? As you will learn in this special edition, our enemy intelligence has improved over the years, enabling us to get a better bead on where the trouble begins. And we have developed stronger weapons, to more precisely pursue and annihilate diseased tissue.

Finding Enemy Forces. Cancer's origins are multifaceted, a combination of an individual's genetic factors and influences from the surrounding environment and his or her personal history and lifestyle. Even stem cells—which, in other contexts, offer promise for the treatment of a variety of ailments—could be to blame. To learn more, turn to page 40 for "Stem Cells: The Real Culprits in Cancer?" by Michael F. Clarke and Michael W. Becker. And in "Mapping the Cancer Genome," on page 22, Francis S. Collins and Anna D. Barker explain how such a tool will help us chart a course across the landscape of human malignancies.

Destroying the Targets. While scientists are grappling to gain a better understanding of cancer's complex beginnings, they also have improved ways of stalling the advance of the disease. In "Taming Vessels to Treat Cancer," on page 64, for instance, Rakesh K. Jain describes how calming the chaos in tumors' blood vessels could facilitate attacking them. Francisco J. Esteva and Gabriel N. Hortobagyi explain how we are also "Gaining Ground on Breast Cancer" with targeted therapies, beginning on page 88.

Hope in the Trenches. Patients with cancer, empowered by expanding informational resources and the changing, more open attitudes of doctors, are living longer and better than ever today, as Lisa Stein writes in "Living with Cancer"; see page 6. Although medicine clearly has much work to do, with advances occurring rapidly, we are on the path to managing this chronic disease.

Mariette DiChristina
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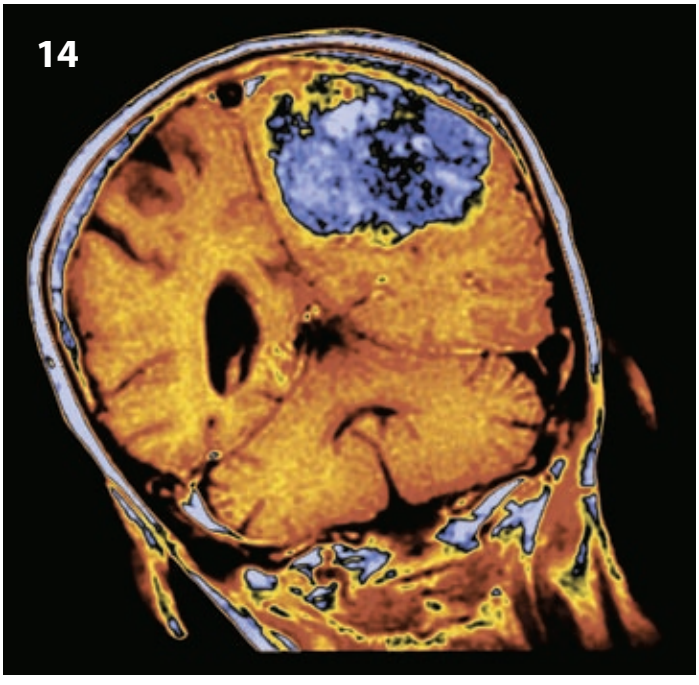

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Cover image by Kenn Brown, Mondolithic Studios.
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Living with Cancer

Keep up your spirits and tap available resources to make the disease manageable

By Lisa Stein

LISA COCCIARDI (*photograph*)

Overview

- Rather than surrendering to despair and impersonal medical treatments, growing numbers of cancer patients are empowering themselves with information and control over their therapies. The trend is finding acceptance in mainstream medicine and helping people with cancer lead healthier lives.
- The experiences of author and filmmaker Kris Carr, who was diagnosed with a rare, incurable malignancy, illustrate how successfully one can manage cancer as a chronic disease.
- The following resource guides offer tips on developing a strategy for managing the illness, asking the right questions of physicians and getting the right professional and personal support.

It was February 2003, and Kris Carr, a photographer and actress, was on a roll. The bubbly, green-eyed stunner was in high demand. She was considered "the Julia Roberts of advertising" (at least according to her agent), thanks to her success in two popular Bud Light commercials that aired during the Super Bowl. She also had some impressive theater and film credits, among them a role in Arthur Miller's *Mr. Peter's Connections,* in which she performed (in the buff, no less) alongside actor Peter Falk.

Like many of her hip young compeers, Carr, then 31, routinely burned the candle at both ends. She existed on energy bars, fast food and coffee downed between nonstop auditions and takes. Every so often her frenetic lifestyle would catch up with her as it did now: she had just returned home to New York City after "partying like a rock star" at Florida's Sarasota Film Festival, where a film she had appeared in premiered, and she was dragging. Time to detox, cleanse her body and soul, exercise and eat right for a spell. She swore off drinking for a month and took a vigorous Jivamukti-style yoga class to kick-start her new get-healthy-quick scheme.

"The following morning I woke up feeling like I was hit by a truck," Carr

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"I DON'T THINK anyone has a better life than me," says cancer patient Kris Carr.

SERGIY TIMASHOV/*Stockphoto (all icons)*

says. Every muscle ached. She dismissed her sore body as a sign that she was more out of shape than she had thought and, as usual, slipped into tight jeans, slathered on a mask of makeup and headed to an audition: a commercial for a diet shake. (She didn't get it: too fat, says the slender onetime model.)

By evening, stiff muscles were the least of Carr's problems. Her pain had worsened, and it was now accompanied by shortness of breath and severe abdominal cramping. She made an appointment to see her doctor the following day.

Gallbladder trouble, the physician surmised after a quick examination. Recommended treatment: yank the pear-shaped organ that, when healthy,

You Have Cancer: Now What?

Diagnosis: cancer. Your head is spinning, and you feel like the wind has been sucked out of you. In a split second, life as you knew it is gone. "Getting diagnosed throws your entire universe into a free fall," Carr writes in her 2007 book *Crazy Sexy Cancer Tips.* "There's no sugarcoating it: cancer is a devastating blow, one that takes time to process."

The first things you should do (after taking a deep breath and trying to chill):

Find the best doctor for your disease: Be willing to travel and *always* get second, third and even fourth opinions to make sure that you're getting the best treatment.

Design a healing plan: Pull together a team of Western physicians as well as integrative doctors (to teach you how to build up your immunity and spiritual grit) to create the best get-healthy recipe. Ask family and friends to chip in and scour the Internet and bookshelves for information. "If you want to heal, you have to take initiative, have a voice and use it," Carr says.

Focus on lifestyle changes: "The only thing that you can control is what you eat, what you drink and how you move," Carr says. She recommends exploring healthy diets, exercise and alternative therapies such as massage, yoga and meditation to boost and maintain your physical and emotional well-being.

Create a support system: "Nobody understands you quite like another cancer survivor," Carr says. "There is incredible strength in that."

Live! "Don't wait for permission to live. Just because you have cancer does not mean that your life is over," Carr insists. "Start living. It's that simple."

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helps the liver flush fats from the body but, when faulty, causes excruciating pain. He gave Carr a prescription for painkillers and sent her for an ultrasound to confirm that her gallbladder was indeed the culprit.

It wasn't.

"When they did the ultrasound, they found the 'lesions.' They could see there were spots all over my liver—so many that it looked like Swiss cheese," Carr says. She was concerned but still blissfully ignorant of the potential ramifications. "I didn't know," she says, "that lesions meant tumors."

A battery of tests over the next few days revealed that Carr was suffering from epithelioid hemangioendothelioma (EHE), a vascular cancer in the lining of the blood vessels in her liver and lungs so rare that only 0.01 percent of the cancer population has it. Around 200 to 300 cases are diagnosed nationwide every year. The cause: unknown. The cancer was stage IV—incurable and inoperable, the doctor said. "Some people say it could have come on like a meteor shower," Carr says; others suspect the tumors had been developing her whole life.

EHE is typically a slow-moving cancer. There are studies under way but currently no cures or definitive treatments. The doctor recommended a "watch and wait" approach. That is, that they take their cues from the tumors—monitor them for two months to gauge whether they were holding steady or moving slowly or swiftly. They were quiet for now, "indolent" in cancer-speak, and the hope was they would stay that way.

It was February 14. "Happy Valentine's Day. You have cancer," Carr wrote in her journal that night.

Questions to Ask

Studies show that cancer (and other) patients who arm themselves with information typically fare better and experience fewer side effects than those who simply follow doctors' orders, no questions asked. Being informed gives them some control over their disease—and that feeling of empowerment plays a role in the healing process. No. 1 rule: do not be cowed by your doctor. Ask him or her to explain anything and everything you don't understand. Prepare questions in advance of appointments (to reduce stress and the odds of forgetting any)—and bring a notebook to jot down answers and other important info. Below are some questions you should ask:

- ❑ What causes this type of cancer?
- ❑ What are the risk factors? If it's genetic, are other family members at risk?
- ❑ What lifestyle changes (diet, exercise, rest) do you recommend?
- ❑ What are my treatment options?
- ❑ Are there activities that should be avoided because they might trigger or exacerbate symptoms?
- ❑ What happens if new symptoms crop up or existing ones worsen?
- ❑ What medical tests or procedures are necessary? How often?
- ❑ What stage is my cancer? What does that mean?
- ❑ What is my overall prognosis or chance of recovery?
- ❑ What are the average survival and cure rates?
- ❑ Could my disease go into remission?
- ❑ What is the recommended treatment?
- ❑ How often will I have to undergo treatment—and for how long?
- ❑ What are the potential side effects?
- ❑ What are the benefits versus the risks of each treatment option?
- ❑ Are there alternative therapies? What are they?
- ❑ What are the expected results of treatment?
- ❑ Is the treatment painful? If so, is there a way to make it more bearable?
- ❑ How long is the recovery? Will it require a hospital stay?
- ❑ When can I resume my normal activity (if it's been curtailed)?
- ❑ Has my cancer spread? If so, how does this change treatment decisions?
- ❑ Am I eligible for any clinical trials?
- ❑ What happens if my disease progresses while I'm in a clinical trial?
- ❑ Who foots the bills if I participate in a clinical trial?
- ❑ Where can I find emotional, psychological and spiritual support?
- ❑ Whom should I call with questions or concerns after office hours?
- ❑ May I contact you or a nurse if I have questions or more symptoms? (If the answer is "no," find another doctor.)

"How could I live

Why Me?

"I FELT LIKE I was punched in the stomach by God," she recalls. "Cancer is such a frightening word. How could this be happening to me? Cancer happened to other people. I was young and vibrant. I was the Bud Girl, for Christ's sake. I felt like I was staring down the barrel of a gun, waiting to find out how many bullets were inside."

There were 24—to be exact—littering her liver and lungs.

Carr pressed the doctor on her options. "Just try and live a normal life," he told her.

With two dozen time bombs ticking

NICHOLAS MONU /*Stockphoto*

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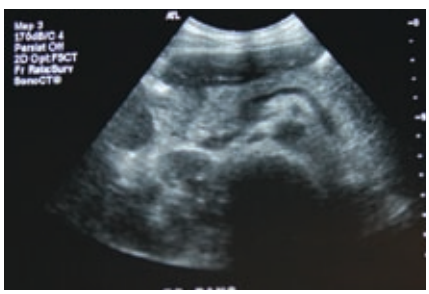
inside her? "How the hell could I do that? How could I live with cancer without thinking of dying every day?" she wondered.

Well, he offered, she *could* try to strengthen her immune system through diet and lifestyle changes.

"He did not know it, but in that moment he planted the seeds for personal revolution," Carr says. "I was not going to kick back and wait for the unknown. I was going to dive in and become a full-time healing junkie."

She set about trying to find out everything she possibly could about cancer. She sought second, third and fourth opinions. "If I had listened to one of the first doctors I talked to, I would have ended up sliced, fried and hauling around not one but three organs that didn't belong to me," she says.



Becoming a "Healing Junkie"

CARR HIT THE BOOKS and the Internet. ("I tell people I have a Ph.D. from Google University," she says, laughing.) She traded in fast food for a vegan diet and swapped martinis for a green brew of cucumbers, kale, celery and sprouts. She formed a "posse" with other young women with cancer. She explored alternative therapies, including massage and meditation, and even spent time in a Zen monastery. And she began the empowering process of documenting and filming her journey—ev-

AFTER DOCTORS found tumors in her liver (*below left*), Carr hit the books to learn more about her disorder.

with cancer without thinking of dying every day?"

Your Odds of Beating Cancer

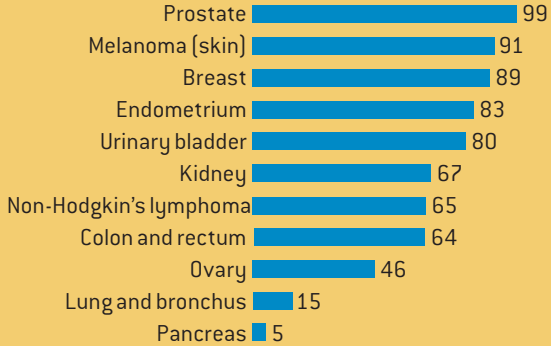
Success in the battle against cancer is often measured in terms of the "five-year relative survival rate." That rate is the number of patients who are still alive five years after being diagnosed, relative to the number who would be expected to survive if they had not come down with the disease. Five years might not seem like a lot, but it is, considering that 67 is the median age for diagnosis.

Below is a sampling of five-year relative survival rates for common types of cancer diagnosed between 1996 and 2004. These rates are calculated by the National Cancer Institute's Surveillance, Epidemiology and End Results (SEER) program, which collects survival data from state registries covering about 26 percent of the U.S. population.

Survival rates have increased dramatically over the years, thanks to earlier detection and better treatments. The five-year relative survival rate for patients diagnosed with any type of cancer in 1975 was 50 percent; the rate jumped to 67 percent in 2000.

Bear in mind that survival rates vary widely depending on the type of cancer and the patient's age, gender, general health, lifestyle and ethnicity. You can find more detailed statistics at http://seer.cancer.gov

Five-Year Survival Rates (percent)



COURTESY OF KRIS CARR (*ultrasound*); BRIAN FASSETT (*Carr*)

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Getting Support: Tips, Tools and Tenderness

You've just been diagnosed with cancer. Now what? First and foremost, do not try to handle this on your own. Allow family and friends to help, and find others in your situation to lean on.

Online resources:

- **www.crazysexycancer.com:** Carr's Web site. Have questions? Want to dish? You can visit her online community, **www.crazysexylife.com**.
- **http://berniesiegelmd.com** and **www.ecap-online.org:** These sites of physician Bernie Siegel, author of *Love, Medicine & Miracles* and *Peace, Love & Healing* (both from Harper Paperbacks, 1990), offer info and tools based on the science of mind-body-spirit medicine.
- **www.cancercare.org:** Need a professional cancer assistant? Try the next best thing. This site is designed to help patients navigate their way through cancer—answering questions, finding help or just "listening" when they need to vent.
- **http://nccam.nih.gov:** The National Center for Complementary and Alternative Medicine of the National Institutes of Health provides information here on alternative and complementary therapies, discoveries and clinical trials.
- **http://hippocrateshealthinstitute.com:** Site of the Hippocrates Health Institute, a world-renowned healing center in Florida.
- **www.mercola.com:** An alternative medicine and education site.
- **www.heardsupport.org:** This site is specifically geared toward patients with hemangioendothelioma, the rare cancer that Carr has.
- **www.livestrong.org:** Site of seven-time Tour de France winner and cancer survivor Lance Armstrong.
- **www.ulmanfund.org:** Provides support programs and resources for patients and their families. Also helpful: a downloadable book penned by founders Doug and Diana Ulman.
- **www.thechinastudy.com:** *The China Study,* by T. Colin Campbell, probes the relationship between diet and cancer and other diseases.
- **www.cancer.gov:** This site of the National Cancer Institute is a comprehensive source of state-of-the-art treatments and clinical trials (including a database of open trials).
- **www.imtooyoungforthis.org:** An invaluable source of support and research for survivors in their 20s and 30s and their families.
- **www.cancersurvivorsunite.org:** Camps and support programs for young adults with cancer.
- **www.youngcancerspouses.org:** A site designed to connect couples dealing with the ups and downs of cancer.
- **www.cancerconsultants.com:** Contains detailed, consumer-friendly information on the latest treatment developments.
- **www.americancancersociety.com:** This American Cancer Society site provides basic information, alternative therapies, ways to manage the disease, and support programs .
- **www.oncolink.com:** This University of Pennsylvania site offers key cancer info and pointers.
- **www.cancerguide.org:** A how-to on researching your disease, searching for clinical trials, and finding out about the latest traditional and alternative therapies.
- **www.cancer.net:** American Society of Clinical Oncology site provides oncologist-approved information to help patients make informed decisions about their health care.
- **www.gildasclub.org:** Named for *Saturday Night Live* comedian Gilda Radner, who died of ovarian cancer, this site provides a support network for patients and their families.
- **www.thewellnesscommunity.org:** The Wellness Community provides support and education for cancer patients and caretakers—and hooks them up with others going through the same thing. It provides info on local wellness communities and even offers a virtual wellness community in Spanish.

erything and everyone she met, from the physicians to the gurus to the quacks. (Beware of quick fixes, she warns: "If anyone offers guarantees—run!")

She conducted her search for an oncologist as though she were CEO of a company that she dubbed Save My Ass Technologies, Inc., treating prospective doctors as though they were job applicants. "If it was the perfect fit: fine," she says. "If not: next!" She nixed some of the candidates for their poor bedside manner ("There should be mutual respect"), others because of their proposed treatment plans. Among the dismissed: the one who recommended a triple organ transplant (her liver and both lungs). "Some doctors are still caught up in the old model of nuke it and cut it out—and sometimes it is really not necessary.... In my case it was not the protocol," Carr says. "Do you want them to be stabbing at you if they're taking that stab in the dark? It's important to make sure you're in the right hands. They can help you, or they can kill you. It's that simple."

The more physicians she interviewed, the more she came to realize that "half the time they don't have the

"We are still

answers," but it is the ones willing to admit that fact who hold the most promise of finding them. Enter the doctor she "hired": George Demetri, director of the Center for Sarcoma and Bone Oncology at the Dana-Farber Cancer Institute in Boston, who, unlike many of the other "job applicants," not only has the medical credentials but, she says, is also "kind and compassionate" and welcomes his patients' input.

Keeping Tumors at Bay

CARR SAYS Demetri believes that she can live her "whole life" with the disease but that it may have to be treated with drugs at some point. "We don't know. There is currently no cure," she notes, "but there's no doubt in my mind that any new information, drugs, and

MANCHAN/GETTY IMAGES

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Medical Resources

Finding a doctor who specializes in cancer care and choosing a treatment facility are essential steps in any patient's recovery program. One good place to start is with the 63 cancer centers that the National Cancer Institute recognizes for "scientific excellence and the capability to integrate a diversity of research approaches" (http://cancercenters.cancer.gov/cancer_centers). You can also check whether the American College of Surgeons' Commission on Cancer (www.facs.org/cancerprogram) approves of a given program. Some of the things to look for in a cancer center include a low mortality index, a high ratio of nurses to patients and opportunities to participate in clinical trials. For more tips, see www.cancer.gov/cancertopics/factsheet/Therapy/doctor-facility. Here is a selection of some of the most respected cancer treatment centers around the country:

Dana-Farber Cancer Institute
Boston
866-408-DFCI
www.dfci.harvard.edu

Memorial Sloan-Kettering Cancer Center
New York City
212-639-2000
www.mskcc.org

Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins
Baltimore
410-502-1033
www.hopkinshospital.org/health_info/Cancer/index.html

Children's Hospital of Philadelphia
215-590-1000
www.chop.edu/consumer/jsp/division/service.jsp?id=26696

Duke Comprehensive Cancer Center
Durham, N.C.
888-ASK-DUKE
www.cancer.duke.edu

University of Texas M. D. Anderson Cancer Center
Houston
877-MDA-6789
www.mdanderson.org

University of Chicago Medical Center
888-UCH-0200
www.uchospitals.edu/specialties/cancer

Mayo Clinic
Rochester, Minn.
(facilities also in Arizona and Florida)
507-284-2511
www.mayoclinic.org/cancer-treatment

University of Washington Medical Center
Seattle
206-598-4100
http://uwmedicine.washington.edu/PatientCare/MedicalSpecialties/SpecialtyCare/UWMEDICALCENTER/Cancer

UCLA Medical Center
Los Angeles
800-UCLA-MD1
www.uclahealth.org

empowered. I have cancer, but I'm dealing with it."

treatment is going to come out of this place [Dana-Farber]. I'm in the right place to be monitored."

Four years after turning the camera on herself, Carr turned her healing journey into a documentary called *Crazy Sexy Cancer,* which TLC bought in the fall of 2006. Last year it had its world premiere at the South by Southwest Film Festival in Austin, Tex.

"I'm not saying that cancer is sexy," she stresses. "What I'm saying is that we are still empowered. We are still alive and whole. I might have cancer, but I'm dealing with it and I'm still all that. The most important thing is to have a voice and use it."

Carr is among a growing num-

HEINZ LINKE *iStockphoto [lab];* LISA COCCIARDI *[Carr]*

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The 411 on Health Insurance

Worried that your health insurance won't cover your treatment? Wondering if you're entitled to disability benefits? These Web sites may help:

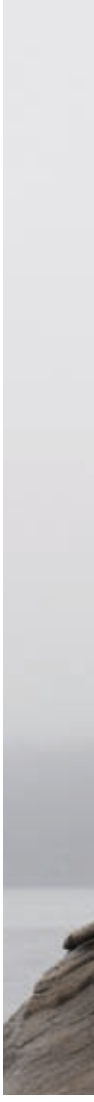
- www.healthinsuranceinfo.net
- www.patientadvocate.org
- www.patient.cancerconsultants.com
- http://cancerguide.org/disability.html
- www.thedisabilityexpert.com
- www.ssa.gov/applyfordisability

ber of people living and thriving with cancer, thanks to medical advances as well as a progressive philosophy in oncology that recognizes past mistakes of overtreatment and welcomes alternative medicine as a partner in the healing process. The new approach, she says, shatters the stigma that cancer is either a death sentence or something that has to be eradicated—and opens the door to treatments designed to keep tumors in check, which could buy time while new therapies are developed. "Many amazing new treatments are targeting tumors and leaving patients with their lives and their immune systems [intact]," she says. "Plus, there is so much that we as patients can do to help our bodies regain health."

Carr is currently developing a nonprofit organization that will work with top oncologists on studies and research using data from the more than 1,000 members of her online community (www.crazysexylife.com) and the 5,000 to 10,000 people who visit her Web site (www.crazysexycancer.com) every week. "We want to be the bridge, one of many bridges, between Western and alternative medicine," she says.

When first diagnosed, Carr viewed cancer as a freight train to death; now she views it as a "catalyst" for change. She changed her lifestyle, met a new community of women and ditched acting for writing, something she never believed she could do. Last year she wrote and published *Crazy Sexy Cancer Tips* (Globe Pequot Press), a book chock-full of practical advice on everything from doctor shopping to diet to how to keep your wits about you when diagnosed with the Big "C" (or any other disease, for that matter). She wrote a companion book, *Crazy Sexy Cancer Survivor: More Rebellion and Fire for Your Healing Journey*, due out in September—and is set to pen a diet and lifestyle manual to be published next year.

Perhaps most important, she says, cancer led her to her "soul mate." She recruited Brian Fassett to help her film, edit and produce her documentary. During the project, they fell in love—and Fassett and Carr (who, when first diagnosed, thought she would never date



"Once I was able to change my focus,

How to Stay Healthy

Patients undergoing treatment can shore up their physical (and emotional) reserves by eating well, exercising and cutting stress (which impairs the immune system). The American Institute for Cancer Research, which funds studies on the role of food and exercise in cancer prevention and treatment, recommends a diet that's at least two-thirds vegetables, fruit, whole grains and beans. Below is a roundup of research related to staying healthy:

- A study of 22,000 healthy Greeks showed their "Mediterranean diet," rich in vegetables, whole grains, olive oil, fruit and fish, reduced their risk of dying from cancer by at least 25 percent. Other studies have found that nutrients in dark, leafy greens may inhibit the growth of tumor cells in breast, skin, lung and stomach cancers and that green tea may thwart cancer development in colon, liver, breast and prostate cells. (A leading theory: flavonoids in tea and carotenoids in leafy greens, which act as antioxidants, may protect against cancer by rooting out free radicals.)
- A pair of 2006 studies showed that regular exercise reduced by up to 61 percent the odds of death in colorectal cancer patients. The findings held even in patients who did not start exercising until after diagnosis.
- A 2005 study showed that 92 percent of nearly 3,000 women with breast cancer who walked or did other exercise three to five hours weekly were still alive 10 years after their diagnosis, compared with 86 percent of those who exercised less than an hour a week.
- A 30-year review of the scientific literature, published in 2004, suggested that cancer patients who feel helpless or who suppress negative emotions may be at greater risk of having their cancer spread than those who play a role in their healing.

PAMELA MOORE (*folders*), JENNY HILL (*runner*) AND VICTOR MELNICIUC (*fruit*) *iStockphoto*

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TODAY Carr says she is "healthier than before I was diagnosed."

BRIAN FASSETT (*Carr*); PAVEL LOSEVSKY *iStockphoto* (*family*)

desperation led to inspiration."

again, let alone marry) got hitched in the fall of 2006. "It was one of the happiest days of my life," she says. "We vowed to be fellow adventurers. We thought it would be way too melodramatic to say 'till death do us part.' This was a day that cancer just was not a part of." They are now considering having kids. ("Will the hormones wake the sleeping dragon? We don't know," she says, "but I refuse to live my life in fear.") And they have started their own production company, Red House Pictures.

So how is the 36-year-old Carr today, more than five years since her life-altering diagnosis? "I am happy and, I think, healthier than I was before I was diagnosed." Her last scan in February showed the tumors are stable.

Looking back on her healing journey, she muses: "The doctors told me to 'watch and wait.' What I prefer is the 'watch and live' approach. I'm not waiting, putting my life on hold. I'm living my life, just with the knowledge that cancer is in my body.

"I think that life is just too sweet to be bitter. Once I was able to change my focus, desperation led to inspiration. I made so many changes, and I thought: This is an awesome life. I mean, honestly, I don't think anyone has a better life than me. How can you live with the knowledge of cancer? I might not ever be able to get rid of it, but I can't let that ruin my life.... I think: Just go for it. Life is a terminal condition. We're all going to die. Cancer patients just have more information, but we all, in some ways, wait for permission to live." SA

Lisa Stein is news editor for Scientific American*'s Web site, www.SciAm.com.*

Looking Ahead: Start a Family?

Does a cancer diagnosis spell the end of your dreams to have a family? In a word—no. Note to readers: check your options *before* undertaking treatments that may cause infertility. In the event that you cannot become pregnant, there is always surrogacy and adoption. Despite what you've heard, it *is* possible to adopt if you've had cancer. The key: pick an agency and country that are open to working with cancer survivors.

For more, check out:

- **www.fertilehope.org:** This site provides unvarnished facts about fertility risks associated with cancer treatment as well as fertility-preservation and parenthood alternatives before, during and after treatment. It outlines the success rates, costs and time requirements for a variety of fertility procedures and also addresses other possibilities, including egg and sperm donation, surrogacy and adoption.
- **www.pregnantwithcancer.org:** This Web site links newly pregnant cancer patients with others with a similar cancer who have already been there, done that.

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EVOLVED FOR CANCER?

BY CARL ZIMMER

Natural selection lacks the power to erase cancer from our species and, some scientists argue, may even have provided tools that help tumors grow

Charles Darwin, 1881

RICHARD MILNER ARCHIVES

NATURAL SELECTION IS NOT NATURAL PERFECTION. Living creatures have evolved some remarkably complex adaptations, but we are still very vulnerable to disease. Among the most tragic of those ills—and perhaps most enigmatic—is cancer. A cancerous tumor is exquisitely well adapted for survival in its own grotesque way. Its cells continue to divide long after ordinary cells would stop. They destroy surrounding tissues to make room for themselves, and they trick the body into supplying them with energy to grow even larger. But the tumors that afflict us are not foreign parasites that have acquired sophisticated strategies for attacking our bodies. They are made of our own cells, turned against us. Nor is cancer some bizarre rarity: a woman in the U.S. has a 39 percent chance of being diagnosed with some type of cancer in her lifetime. A man has a 45 percent chance.

NATURAL SELECTION HAS FAVORED CERTAIN DEFENSES AGAINST CANCER BUT CANNOT ELIMINATE IT ALTOGETHER.

These facts make cancer a grim yet fascinating puzzle for evolutionary biologists. If natural selection is powerful enough to produce complex adaptations, from the eye to the immune system, why has it been unable to wipe out cancer? The answer, these investigators argue, lies in the evolutionary process itself. Natural selection has favored certain defenses against cancer but cannot eliminate it altogether. Ironically, natural selection may even inadvertently provide some of the tools that cancer cells can use to grow.

The study of cancer evolution is still in its infancy, with much debate about the mechanisms involved and much testing of hypotheses left to carry out. Some medical researchers remain skeptical that the work will affect the way they fight the disease. Evolutionary biologists agree that they are not about to discover a cure for cancer, but they argue that understanding cancer's history could reveal clues that would otherwise remain hidden. "Obviously, we always have that in the back of our minds in everything we do," says Judith Campisi of Lawrence Berkeley National Laboratory.

The Dawn of Cancer

AT ITS ROOT, cancer is a disease of multicellularity. Our single-celled ancestors reproduced by dividing in two. After animals emerged, about 700 million years ago, the cells inside their bodies continued to reproduce by dividing, using the molecular machinery they inherited from their progenitors. The cells also began to specialize as they divided, forming different tissues. The complex, multicellular bodies animals have today were made possible by the emergence of new genes that could control how cells divided—such as by stopping the cells' reproduction once an organ reached its adult size. The millions of animal species are evidence of the great evolutionary success that came with acquiring a body. But bodies also present a profound risk. Whenever a cell inside a body divides, its DNA has a small chance of acquiring a cancer-causing mutation. "Every time a cell divides, it's going to be at risk of developing into cancer," Campisi says.

Rare mutations, for instance, may cause a cell to lose restraint and begin to multiply uncontrollably. Other mutations can add to the problem: They may allow deranged cells to invade surrounding tissues and spread through the body. Or they may allow tumor cells to evade the immune system or attract blood vessels that can supply fresh oxygen.

Cancer, in other words, re-creates within our own bodies the evolutionary process that enables animals to adapt to their environment. At the level of organisms, natural selection operates when genetic mutations cause some organisms to have more reproductive success than others; the mutations get "selected" in the sense that they persist and become more common in future generations. In cancer, cells play the role of organisms. Cancer-causing changes to DNA cause some cells to reproduce more effectively than ordinary ones. And even within a single tumor, more adapted cells may outcompete less successful ones. "It's like Darwinian evolution, except that it happens within one organ," explains Natalia Komarova of the University of California, Irvine.

Limits to Defenses

ALTHOUGH OUR BODIES may be vulnerable to cancer, they also have many ways to halt it. These strategies probably resulted from natural selection, because mutations that made our ancestors less likely to die of cancer in their prime could have raised their reproductive success. But given the many millions of people who get cancer every year, it is obvious that these defenses have not eradicated the disease. By studying the evolution of these defenses, biologists are trying to understand why they fall short.

Tumor suppressor proteins are among the most effective defenses against cancer. Studies suggest that some of these proteins prevent cancer by monitoring how a cell reproduces. If the cell multiplies in an abnormal way, the proteins induce it to die or to slip into senescence, a kind of early retirement. The cell survives, but it can no longer divide.

Overview/*Cancer Evolution*

- Natural selection has only a limited ability to prevent cancer. It has provided some defenses, but these tend to delay the disease until late in life rather than eliminating it entirely.
- In addition, evolutionary forces have apparently favored some genes that can contribute to cancer's development or aggressiveness.
- An understanding of cancer's evolutionary history—and how individual tumors evolve in the body—could suggest fresh angles of attack on the disorder.

Tumor suppressor proteins play a vital role in our survival, but scientists have recently discovered something strange about them: in some respects, we would be better off without them.

Norman E. Sharpless of the University of North Carolina at Chapel Hill genetically engineered mice to study the effect of one of these proteins, called p16 (or, more properly, p16-Ink4a). He and his colleagues created a line of mice that lacked a functional gene for p16 and thus could not produce the protein. In September 2006 the group published three studies on the mice. As expected, the animals were more prone to cancer, which could arise when they were only a year old.

But losing the p16 gene had an upside. When the mice got old, their cells still behaved as if they were young. In one experiment, the scientists studied older mice, some of which had working p16 genes and some of which did not. They destroyed insulin-producing cells in the pancreases of the animals. The normal rodents could no longer produce insulin and developed fatal diabetes. But the ones without the p16 protein developed only mild diabetes and survived. The progenitors of their insulin-producing cells could still multiply quickly, and they repopulated the pancreas with new cells. The scientists found similar results when they examined cells in the blood and brains of the mice: p16 protected them against cancer but also made them old.

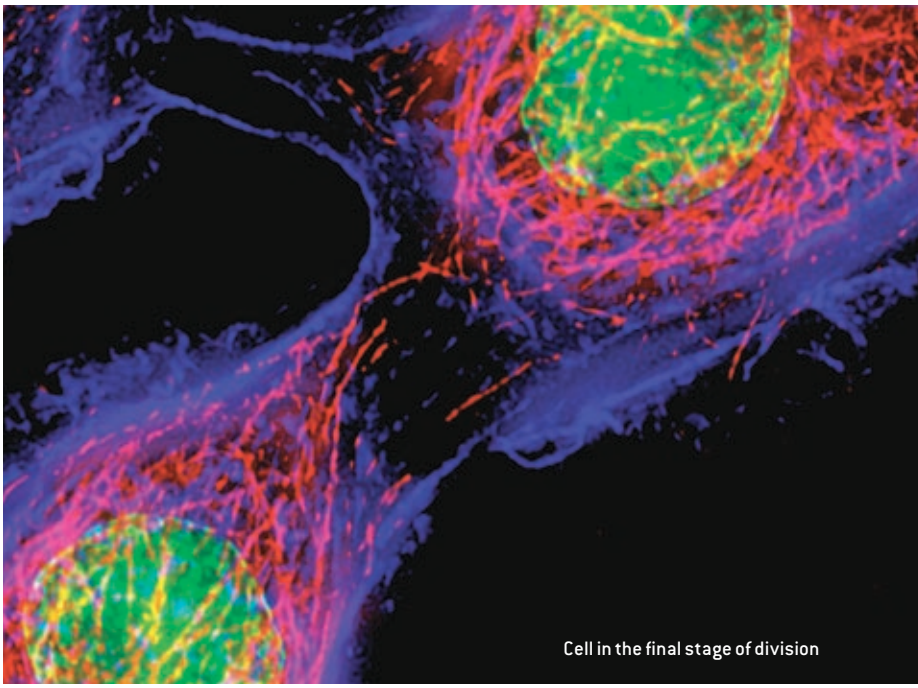
These results support a hypothesis Campisi has developed over the past few years. Natural selection favors anticancer proteins such as p16, but only in moderation. If these proteins become too aggressive, they can create their own threats to health by making bodies age too quickly. "It's still a working hypothesis," Campisi admits, "but the data are looking stronger and stronger."

Delaying the Inevitable

A DEFENSE AGAINST CANCER does not have to eradicate the disease completely to be favored by natural selection. If it can just delay tumors until old age, it may allow people to have more children, on average, than others who lack the defense. It may seem cruel for evolution to stick old people with cancer, but as Jarle Breivik of the University of Oslo points out, "natural selection does not favor genes because they let us live long and happy lives. They are selected for their ability to propagate their information through the generations."

Anticancer proteins such as p16 may favor the young over the old. When p16 pushes a cell into senescence, the cell does not just stop multiplying. It also begins producing an odd balance of proteins. Among the proteins it makes is vascular endothelial growth factor (VEGF), which triggers the growth of more blood vessels. VEGF fosters the growth of tumors by supplying them with extra nutrients. In young people, p16's main effect may be to suppress cancerous cells. But over time, it may create a growing population of senescent cells, which could make people more vulnerable to cancer in old age.

"EVERY TIME A CELL DIVIDES, IT'S GOING TO BE AT RISK OF DEVELOPING INTO CANCER."
—Judith Campisi

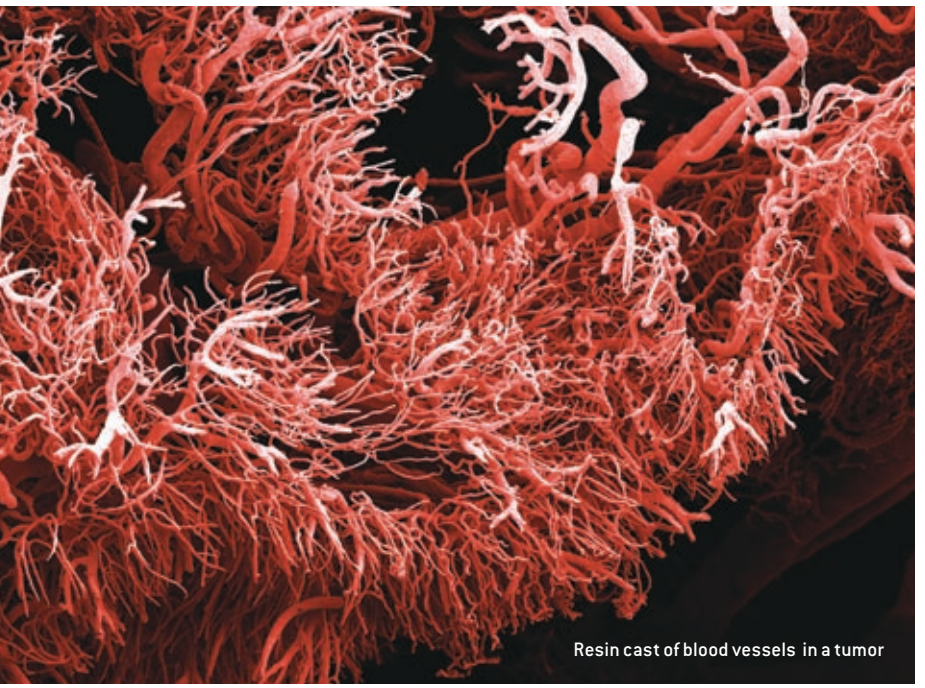


Cell in the final stage of division

PAUL ANDREWS University of Dundee/Photo Researchers, Inc.

Another way to delay cancer is to set up several lines of defense. Studies on colon cancer, for example, show that cells in the colon must acquire mutations to several genes before they turn cancerous. These defense lines do not prevent people from getting colon cancer—in fact, it is the third most common form of the disease. But the need for multiple mutations to occur in a cell may reduce the chances that colon cancer will arise in young individuals. The average age of people diagnosed with colon cancer is 70.

Not all cancers strike the old, of course. Most victims of a cancer of the retina called retinoblastoma, for example, are children. But Leonard Nunney of the University of



Resin cast of blood vessels in a tumor

THE ABILITY TO STIMULATE NEW BLOOD VESSEL FORMATION SERVES A TUMOR JUST AS IT DOES A PLACENTA.

California, Riverside, argues that evolution is responsible for that difference between the two cancers. He points out that colon cells have many more opportunities for acquiring dangerous mutations than retinal cells do. The colon is a large organ made of many cells, which continue replicating throughout a person's life as old cells slough off and new ones take their place. That risk puts a big evolutionary premium on defenses that can prevent colon cells from turning cancerous.

The retina, on the other hand, is "the smallest bit of tissue you can imagine," as Nunney puts it. That small set of retinal cells also stops multiplying by the time a child turns five. With fewer cell divisions occurring, the retina has far fewer opportunities to turn cancerous. As a result, retinoblastoma is extremely rare, striking only four people in a million. Because the risk is so much lower, Nunney argues, natural selection cannot drive the spread of new defenses against retinoblastoma. A defense against cancer in the retina would make very little difference to the average reproductive success of a population.

THE AUTHOR

CARL ZIMMER writes frequently about evolution for the *New York Times, National Geographic* and other publications. He is the author of six books, including *Parasite Rex* and *Soul Made Flesh*. His latest book is *Microcosm:* E. coli *and the New Science of Life* (Pantheon, 2008). His blog, The Loom (www.scienceblogs.com/loom), is a winner of *Scientific American*'s Science and Technology Web Awards.

Making Tools for Tumors

RECENT RESEARCH SUGGESTS that natural selection may have altered genes in ways that make cancer cells more dangerous. Evolutionary biologists discovered this disturbing possibility as they searched for the changes that have made us uniquely human. After our ancestors diverged from other apes about six million years ago, they experienced natural selection as they adapted to a new way of life as a toolmaking, savanna-walking hominid. Scientists can distinguish between genes that have not changed significantly since the origin of hominids and those that have undergone major alteration as a result of selection pressures. It turns out that among the genes that have changed most dramatically are some that play important roles in cancer.

Scientists suspect that the adaptive advantages brought by these genes outweigh the harm they may cause. One of these highly evolved cancer genes makes a protein called fatty acid synthase (FAS). Normal cells use the protein encoded by this gene to make some of their fatty acids, which are used for many functions, such as building membranes and storing energy. In tumors, however, cancer cells produce FAS protein at a much higher rate. The protein is so important to them that blocking the activity of the gene can kill cancer cells. By comparing the sequence of the FAS gene in humans and other mammals, Mary J. O'Connell of Dublin City University and James McInerney of the National University of Ireland found that the gene has undergone strong natural selection in humans. "This gene has really changed in our lineage," McInerney says.

McInerney cannot say what FAS does differently in humans, but he is intrigued by a hypothesis put forward by the late psychiatrist David Horrobin in the 1990s. Horrobin argued that the dramatic increase in the size and power of the human brain was made possible by the advent of new kinds of fatty acids. Neurons need fatty acids to build membranes and make connections. "One of the things that might allow a larger brain size was our ability to synthesize fats," McInerney speculates. But with that new ability may have come a new tool that cancer cells could borrow for their own ends. Cancer cells may, for example, use FAS as an extra source of energy.

Many fast-evolving cancer genes normally produce proteins in tissues involved in re-

CLOUDS HILL IMAGING LTD. *Photo Researchers, Inc.*

production—in the placenta, for instance. Bernard J. Crespi of Simon Fraser University in British Columbia and Kyle Summers of East Carolina University argue that these genes are part of an evolutionary struggle between children and their mothers.

Natural selection favors genes that allow children to draw as much nourishment from their mothers as possible. A fetus produces the placenta, which grows aggressively into the mother's tissue and extracts nutrients. That demand puts the fetus in conflict with its mother. Natural selection also favors genes that allow mothers to give birth to healthy children. If a mother sacrifices too much in the pregnancy of one child, she may be less likely to have healthy children afterward. So mothers produce compounds that slow down the flow of nutrients into the fetus.

Each time mothers evolve new strategies to restrain their fetuses, natural selection favors mutations that allow the fetuses to overcome those strategies. "It's a restrained conflict. There's a tug-of-war about how much the fetus is going to take from the mother," Crespi says.

Genes that allow cells to build a better placenta, Crespi and Summers argue, can get hijacked by cancer cells—turned on when they would normally be silent. The ability to stimulate new blood vessel formation and aggressive growth serves a tumor just as it does a placenta. "It's something naturally liable to be co-opted by cancer cell lineages," Summers says. "It sets up the opportunity for mutations to create tools for cancer cells to use to take over the body."

Yet even though activation of these usually quiet genes may make cancers more potent, natural selection may still have favored them because they helped fetuses grow. "You may get selection for a gene variant that helps the fetus get a little more from mom," Crespi says. "But then, when that kid is 60, it might increase the odds of cancer by a few percent. It's still going to be selected for because of the strong positive early effects."

Sperm are another kind of cell that multiplies rapidly. But whereas placental cells proliferate for a few months, sperm-making cells function for a lifetime. "For decades, human males are producing an enormous amount of sperm all the time," says Andrew Simpson of the Ludwig Institute for Cancer Research in New York City. Genes that operate specifically in such cells are also among the fastest evolving in the human genome. A gene that allows a progenitor sperm cell to divide faster than other cells will become more common in a man's population of sperm. That means it will be more likely to get into a fertilized egg and be passed down to future generations.

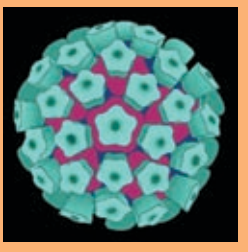
Unfortunately for us, genes that make for fast-breeding sperm cells can make for fast-

LINDA M. STANNARD *University of Cape Town/Photo Researchers, Inc.*

Evolution of a Cancer-Causing Virus

The American Cancer Society estimates that 17 percent of all cancer cases—more than 1.8 million a year—are caused by viruses and other infectious agents. Scientists are studying the evolution of these cancer-causing pathogens to find hints for fighting them. One such pathogen is the human papillomavirus, responsible for most of the half a million cases of cervical cancer diagnosed annually. The virus can cause host cells to divide long after normal cells would stop and also prevents them from repairing mutations to their DNA.

Scientists have reconstructed some of the virus's evolutionary history by sequencing and comparing the genomes of hundreds of different types of viruses. Papillomaviruses, which form a large family, are found in most vertebrates, in whom they typically engender only warts and other benign growths. Yet when *Homo sapiens* first emerged—about 200,000 years ago in Africa—our ancestors already carried a number of strains that could infect our species and no other animal, and these included cancer-causing types.



HUMAN PAPILLOMAVIRUS is shown in a computer-generated rendering.

After about 100,000 years, *H. sapiens* expanded out of Africa to other continents, bringing the viruses with them. As human populations became isolated from one another, their papillomaviruses did as well. Consequently, the genealogy of human papillomaviruses reflects human genealogy. The oldest lineage of the viruses is most common in living Africans, for example. Native Americans descended from Asians, and their viruses share that kinship.

This coevolution may be medically relevant, because the viruses appear to have adapted to their hosts. In August 2006 scientists published a report in the *Journal of the National Cancer Institute* on the persistence of various virus types in different ethnic groups. A woman who becomes infected by a virus having an ancient association with her ethnic group will carry the virus for a longer time than if she were infected by another type.

Scientists are also investigating how certain benign papillomaviruses evolved to cause cancer. Their discoveries will become all the more important as vaccines are introduced against the viruses. The FDA has approved a vaccine against the most dangerous human papillomavirus strain, known as H16. But evolutionary studies indicate that on rare occasions, human papillomavirus types have traded genes involved in triggering cancer. The global HIV epidemic might raise the risk of this gene swapping. As HIV weakens a person's immune system, more types of human papillomaviruses can invade and coexist. This mingling could conceivably give rise to a new cancer-causing strain for which today's vaccines would be less protective. —*C.Z.*

breeding cancer cells. Normally, nonsperm cells prevent these genes from making proteins. "These are genes that need to be firmly silenced, because they are dangerous genes," Simpson says. It appears that in cancer cells, mutations can unlock these sperm genes, allowing the cells to multiply quickly.

How vs. Why

EVOLUTIONARY BIOLOGISTS hope that their research can help in the fight against cancer. In addition to clarifying why evolution has not eradicated cancer, evolutionary biology may elucidate one of the most daunting challenges faced by oncologists: the emergence of drug-resistant tumors.

Chemotherapy drugs often lose their effectiveness against cancer cells. The process has many parallels to the evolution of resistance to antiviral drugs in HIV. Mutations that allow cancer cells to survive exposure to chemotherapy drugs enable the tumor cells to outcompete more vulnerable cells. Understanding the evolution of HIV and other pathogens has helped scientists to come up with new strategies for avoiding resistance. Now scientists are investigating how understanding the evolution within tumors could lead to better ways of using chemotherapy.

The concepts evolutionary biologists have been exploring are relatively new for most cancer biologists. Some are reacting with great enthusiasm. Simpson believes, for instance, that deciphering the rapid evolution of sperm-related genes could help in the fight against tumors that borrow them. "I think it's absolutely crucial to understand exactly why there is such strong selection on these genes," Simpson says. "Understanding that will give us a real insight into cancer."

Bert Vogelstein of the Howard Hughes Medical Institute also finds it useful to view cancer through an evolutionary lens. "Thinking about cancer in evolutionary terms jibes perfectly with the views of cancer molecular geneticists," he says. "In one sense, cancer is a side effect of evolution."

But Vogelstein is not yet persuaded by the significance of fast-evolving cancer genes: "One has to be a little cautious. The first question I would ask is, Are they looking at the whole genome in a wholly unbiased way?" McInerney acknowledges that such systematic studies have not yet been conducted, but the early results have prompted him and other scientists to begin them.

Some cancer specialists are leery of the entire approach. Christopher Benz of the Buck Institute for Age Research in Novato, Calif., says that no insights from evolution should be accepted until they are put to an experimental test the way any other hypothesis would be. "Call me skeptical," he says.

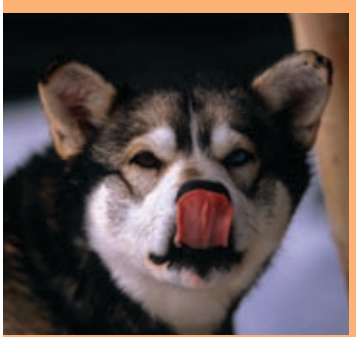
Crespi is familiar with this skepticism, and he thinks that it may emerge from the different kinds of questions evolutionary biologists and cancer biologists ask. "The people working on cancer are working on the

DANIEL BOSCHUNG *zefa/Corbis*

The Surprising History of a Dog Cancer

A canine cancer called Sticker's sarcoma can be transmitted both through sex and by licking or touching a tumor. Once established in a new host, it can produce tumors that grow to the size of grapefruits before gradually disappearing. Many scientists once thought that the disease, like cervical cancer, was spread by viruses. Now they know that the cancer cells themselves move from dog to dog and have been spreading this way for centuries.

A team of scientists from University College London and the University of Chicago analyzed the genes of Sticker's sarcoma cells collected from dogs around the world. They found that the tumors are much more genetically similar to one another than they are to the dogs in which they grew. Additional research confirmed that the tumors belong to a single lineage of cancer cells.

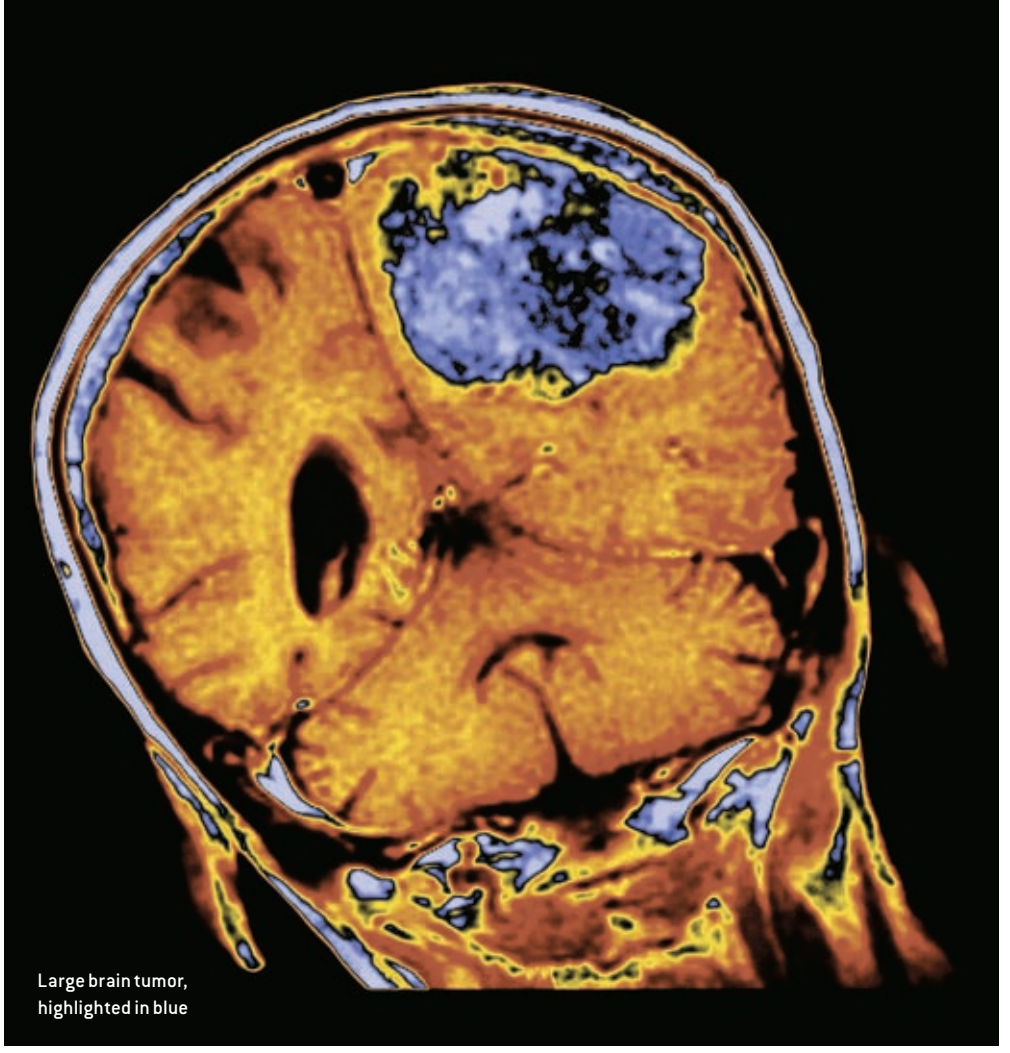


SOME HUSKY might have started the spread of Sticker's sarcoma cells among dogs hundreds of years ago.

"It represents the evolution of a cancer cell into a successful parasite of worldwide distribution," the scientists wrote in 2006 in the journal *Cell*.

Investigators have identified only a few other possible examples of parasitic cancer. Tasmanian devils, for example, can spread a facial tumor by biting one another. Why aren't there more parasitic cancers? Organ transplantation may offer a clue. One of the biggest dangers in organ transplantation is rejection, in which a patient's immune system violently attacks the organ. All vertebrates reject grafts of foreign tissue with this kind of ferocity. It is possible that this rejection response evolved hundreds of millions of years ago as a defense against parasitic cancers.

Sticker's sarcoma appears to have evolved its way around this ancient defense. The cells in the tumor make very few of the surface proteins that vertebrates use to distinguish self from nonself—allowing them to evade an all-out attack from the dog's immune system. Instead the immune system erodes the tumor slowly over the course of several months, and individual cancer cells can survive even after the tumor is gone. Rather than being just an ordinary cancer that dies with its host, it has become a cancer that can live for centuries. —*C.Z.*



Large brain tumor, highlighted in blue

SIMON FRASER *RNC, Newcastle upon Tyne/Photo Researchers, Inc.*

how question, and the evolution people are working on the why," he says.

Perhaps by asking different questions, evolutionary biologists will be able to contribute to some of the debates among cancer biologists. One long-standing argument focuses on whether mice are good models for cancer in humans. Some evolutionary biologists argue that they are not, because of their separate history. Rodents inherited the same set of genes as we did from our common ancestor some 100 million years ago, but then many of those genes underwent more change in the two lineages. Cancer-related genes such as FAS may have experienced intense evolutionary change in humans in just the past few million years, making them significantly different from their counterparts in mice.

Mice may also be a poor choice for a cancer model because of the way they reproduce. Scientists have bred lab mice to produce more pups at a faster rate than their wild cousins. Such manipulation may have altered the evolutionary trade-off faced by mice, so that they are rewarded for investing energy into growing quickly and reproducing young. At the same time, this artificial selection may be selecting against cancer defenses. "We have changed their life histories by selecting on their timing of reproduction," Crespi says.

Ultimately, the study of the evolution of cancer may reveal why eradicating the disease has proved so difficult. "There is no real solution to the problem," Breivik says. "Cancer is a fundamental consequence of the way we are made. We are temporary colonies made by our genes to propagate them to the next generation. The ultimate solution to cancer is that we would have to start reproducing ourselves in a different way." SA

ULTIMATELY, STUDY OF THE EVOLUTION OF CANCER MAY REVEAL WHY ERADICATING THE DISEASE HAS PROVED SO DIFFICULT.

MORE TO EXPLORE

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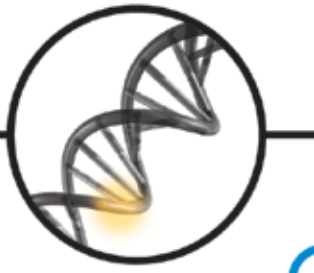
MAPPING THE CANCER GENOME

Pinpointing the genes involved in cancer will help chart a new course across the complex landscape of human malignancies



By Francis S. Collins and Anna D. Barker

"If we wish to learn more about cancer, we must now concentrate on the cellular genome." Nobel laureate Renato Dulbecco penned those words more than 20 years ago in one of the earliest public calls for what would become the Human Genome Project. "We are at a turning point," Dulbecco, a pioneering cancer researcher, declared in 1986 in the journal *Science*. Discoveries in preceding years had made clear that much of the deranged behavior of cancer cells stemmed from damage to their genes and alterations in their functioning. "We have two options," he wrote. "Either try to discover the genes important in malignancy by a piecemeal approach, or … sequence the whole genome."

Dulbecco and others in the scientific community grasped that sequencing the human genome, though a monumental achievement itself, would mark just the first step of the quest to fully understand the biology of cancer. With the complete sequence of nucleotide bases in normal human DNA in hand, scientists would then need to classify the wide array of human genes according to their function—which in turn could reveal their roles in cancer. Over the span of two decades Dulbecco's vision has moved from pipe dream to reality. Less than three years after the Human Genome Project's completion, the National Institutes of Health officially launched the pilot stage of an effort to create a comprehensive catalogue of the genomic changes involved in cancer: The Cancer Genome Atlas (TCGA).

The main reason to pursue this next ambitious venture in large-scale biology with great urgency is cancer's terrible toll on humankind. Every day more than 1,500 Americans die from cancer—about one person every minute. As the U.S. population ages, this rate is expected to rise significantly in the years ahead unless investigators find ways to accelerate the identification of new vulnerabilities within cancerous cells and develop novel strategies for attacking those targets.

Still, however noble the intent, it takes more than a desire to ease human suffering to justify a research enterprise of this magnitude. When applied to the 50 most common types of cancer, this effort could ultimately prove to be the equivalent of more than 10,000 Human Genome Projects in terms of the sheer volume of DNA to be sequenced. The dream must therefore be matched with an ambitious but realistic assessment of the emerging scientific opportunities for waging a smarter war against cancer.

A Disease of Genes

THE IDEA THAT ALTERATIONS to the cellular genome lie at the heart of all forms of cancer is not new. Since the first identification in 1981 of a cancer-promoting version of a human gene, known as an oncogene, scientists have increasingly come to understand that cancer is caused primarily by mutations in specific genes. The damage can be incurred through exposure to toxins or radiation, by faulty DNA repair processes or by errors that occur when DNA is copied prior to cell division. In relatively rare cases, a cancer-predisposing mutation is carried within a gene variant inherited from one's ancestors.

Whatever their origin, these mutations disrupt biological pathways in ways that result in the uncontrolled cell replication, or growth, that is characteristic of cancer as well as other hallmarks of malignancy, such as the abil-

SLIM FILMS

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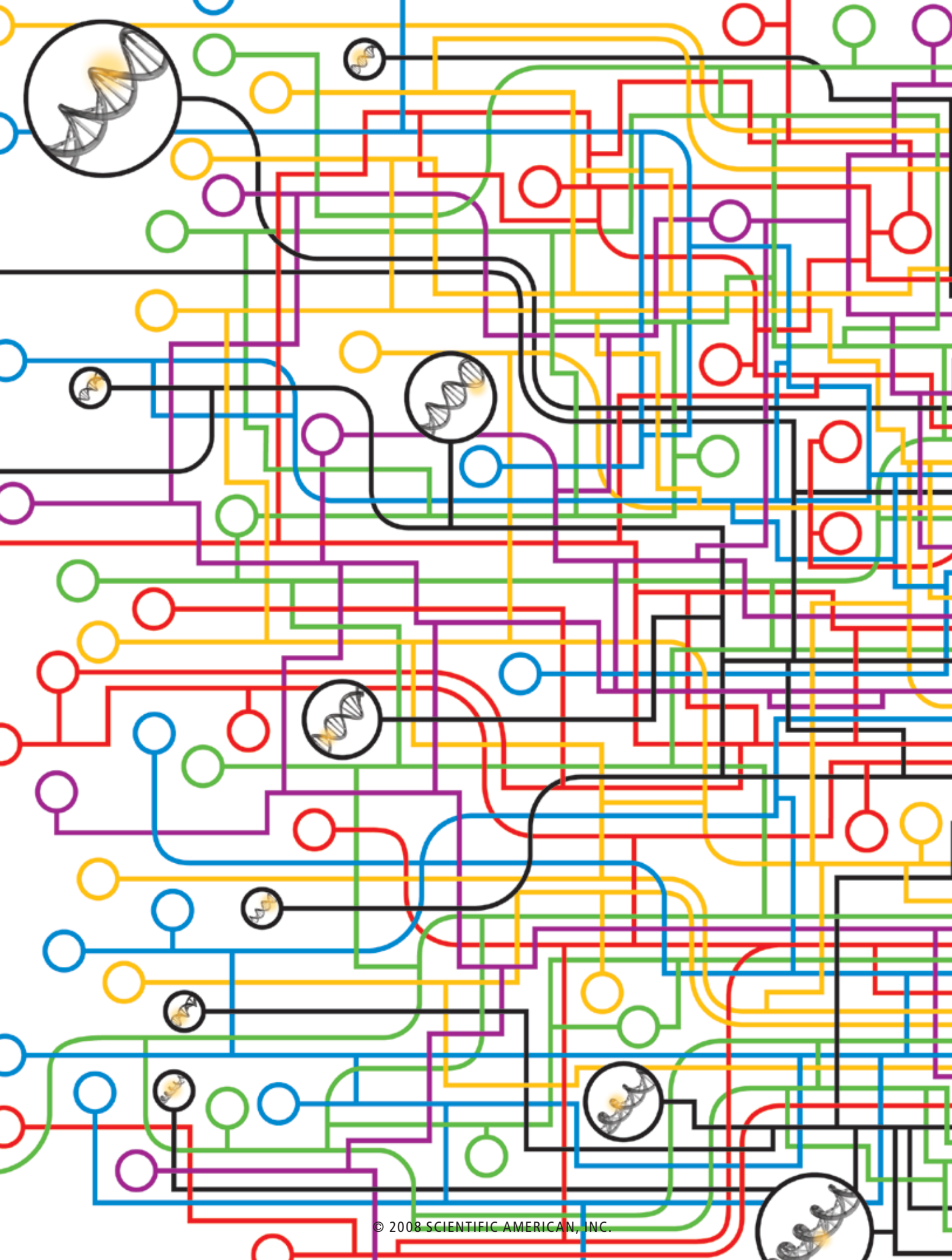

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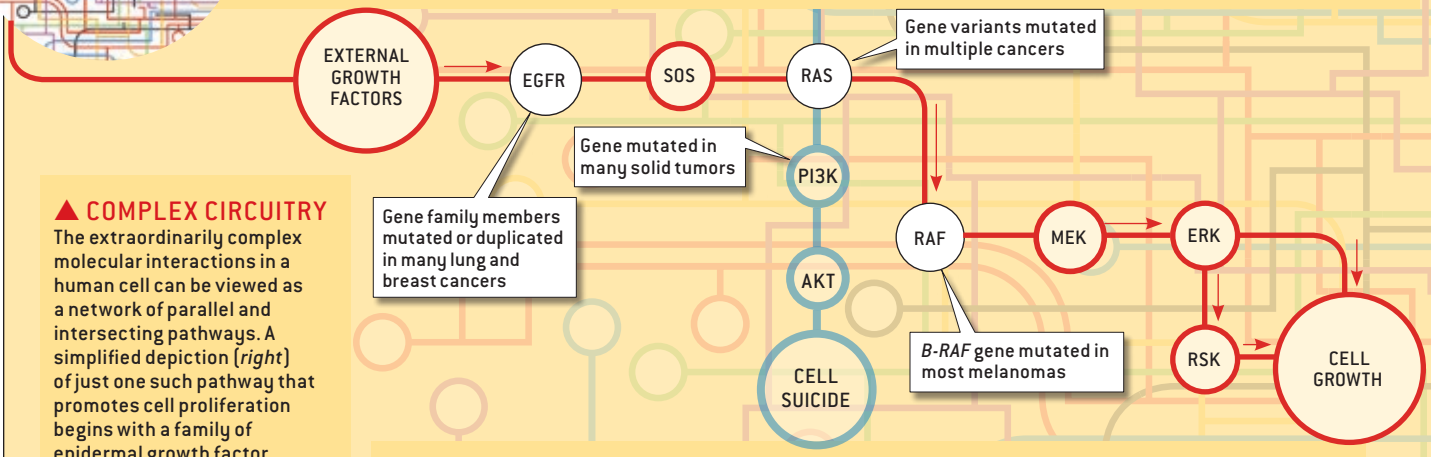
MANY PATHWAYS TO MALIGNANCY

Gene malfunctions underlie the ability of cancer cells to escape normal constraints on a cell's behavior. Because genes give rise to proteins that serve as cellular building blocks, signals and regulators of other genes, a mutation that disables one gene, or causes it to be overactive, can have multiple deranging effects on the cell (*below*). Nevertheless, cells usually need to accrete several cancer-promoting, or oncogenic, mutations in separate genes to acquire the hallmark properties of malignancy (*box at right*). Identifying all the genes whose alteration can produce those traits should one day reveal which mutations are the true drivers of specific types of cancer—even of a specific patient's malignancy—and therefore the most effective ways to intervene in the disease.



▲ COMPLEX CIRCUITRY

The extraordinarily complex molecular interactions in a human cell can be viewed as a network of parallel and intersecting pathways. A simplified depiction (*right*) of just one such pathway that promotes cell proliferation begins with a family of epidermal growth factor receptors (EGFR) in the cell membrane. Their stimulation by growth factors outside the cell transmits signals to additional proteins and genes, ultimately prompting the cell to "grow" by dividing.

▲ ONCOGENIC MUTATIONS

In a significant portion of lung and breast tumors, members of the *EGFR* gene family are mutated or duplicated, which boosts the number or function of the receptors they encode, overstimulating this growth pathway. Damage to downstream genes can have similar results. Changes in the *B-RAF* gene, seen in some 70 percent of melanomas, also promote hyperactive cell proliferation. Versions of the *RAS* gene are mutated in many cancer types, which can affect cell growth as well as intersecting pathways—for example, interfering with a suicide program that normally destroys damaged cells.

ity to invade neighboring tissues and to spread to sites throughout the body. Some mutations may disable genes that normally protect against abnormal cell behavior, whereas others increase the activity of disruptive genes. Most cells must acquire at least several of these alterations before they become transformed into cancer cells—a process that can take years.

Over the past two decades many individual research groups have used groundbreaking molecular biology techniques to search for mutations in genes that are likely candidates for wreaking havoc on normal patterns of cell growth and behavior. By early 2007 such small-scale approaches had identified 350 cancer-related genes and yielded many significant insights into this diabolical disease. A database of these changes, called the catalogue of somatic mutations in cancer, or COSMIC, is maintained by Michael Stratton's group at the Wellcome Trust Sanger Institute in Cambridge, England. But no one imagines that it is the complete list.

So does it make sense to continue exploring the genomic basis of cancer at cottage-industry scale when we now possess the means to vastly increase the scope and speed of discovery? In recent years a number of ideas, tools and technologies have emerged and, more important, converged in a manner that has convinced many leading minds in the cancer and molecular biology communities that it is time for a systematic, collaborative and comprehensive exploration of the genomics of cancer.

The Human Genome Project laid a solid foundation for TCGA by creating a standardized reference sequence of the three billion DNA base pairs in the genome of normal human tissues. Now another initiative is needed to compare the DNA sequences and other physical traits of the genomes of normal cells with those of cancerous cells, to identify the major genetic changes that drive the hallmark features of cancer [*see box on opposite page*]. The importance of international partnerships in large-scale biology to pool resources and speed scientific discovery was also demonstrated by the Human Genome Project, and TCGA is exploring similar collaborations.

Finally, the Human Genome Project spurred significant

Overview/*Cancer Connections*

- Changes in the structure or activity of genes underlie the malignant behavior of cancer cells.
- Identification of genes involved in certain cancers is already advancing diagnosis and treatment.
- The Cancer Genome Atlas is a monumental initiative to eventually identify all the genetic alterations in different forms of cancer so that gene changes driving the disease can be targeted directly.

LUCY READING-IKKANDA; ACKNOWLEDGMENT: SPECIAL THANKS TO JEFFREY SETTLEMAN OF THE CENTER FOR CANCER RESEARCH AT MASSACHUSETTS GENERAL HOSPITAL AND DAPHNE W. BELL OF THE NHGRI CANCER GENETICS BRANCH FOR THEIR ADVICE ON THE PATHWAY ILLUSTRATION

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Hallmarks of Cancer

The six abnormal capabilities listed below together give tumors their lethal power to overrun their native tissue and spread through the body.

Self-sufficiency in growth signaling
Cancer cells amplify external growth cues or generate their own.

Insensitivity to antigrowth signals
Cancer cells become deaf to quiescence cues from surrounding tissue.

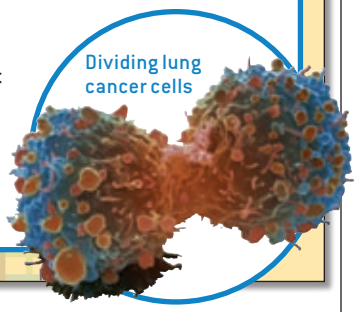
Evasion of cell suicide
Mechanisms that should trigger or carry out a self-destruct program in damaged cells are disabled or overridden.

Limitless replicative potential
Cancer cells evade intrinsic limits on the number of times a normal cell can divide.

Sustained blood vessel growth
Tumors emit signals promoting the development of new blood vessels to deliver oxygen and nutrients.

Invasiveness and motility
Cancer cells defy multiple signals and forces that hold a cell in place and prevent it from traveling to—and thriving in—other tissues.

Adapted from "The Hallmarks of Cancer," by Douglas Hanahan and Robert A. Weinberg, in Cell, *Vol. 100; January 7, 2000.*


Dividing lung cancer cells

advances in the technologies used to sequence and analyze genomes. At the start of that project in 1990, for example, the cost of DNA sequencing was more than $10 per "finished" nucleotide base. Today the cost is less than a penny per base and is expected to drop further with the emergence of innovative sequencing methods. Because of these and other technological developments, the large-scale approach embodied in TCGA—unthinkable even a few years ago—has emerged as perhaps the most efficient and cost-effective way to identify the wide array of genomic factors involved in cancer.

Proofs of Concept

PILES OF DATA are, of course, not worth much without evidence that comprehensive knowledge of cancer's molecular origins can actually make a difference in the care of people. Several recent developments have provided proofs of concept that identifying specific genetic changes in cancer cells can indeed point to better ways to diagnose, treat and prevent the disease. They offer encouraging glimpses of what is to come and also demonstrate why the steps toward those rewards are complex, time-consuming and expensive.

In 2001, when the Wellcome Trust Sanger Institute began its own effort to use genomic technologies to explore cancer, the project's immediate goal was to optimize robotics and information management systems in test runs that involved sequencing 20 genes in 378 cancer samples. But the investigators hit pay dirt a year later when they found that a gene called *B-RAF* was mutated in about 70 percent of the malignant melanoma cases they examined. A variety of researchers swiftly set their sights on this potential new therapeutic target in the most deadly form of skin cancer. They tested multiple approaches—from classic chemical drugs to small interfering ribonucleic acids—in cell lines and in mice, to see if these interventions could block or reduce the activity of *B-RAF* or inhibit a protein called MEK that is overproduced as a result of *B-RAF* mutations. Today the most promising of these therapies are being tested in clinical trials.

Other research groups have already zeroed in on genetic mutations linked to certain types of breast cancer, colon cancer, leukemia, lymphoma and additional cancers to develop molecular diagnostics, as well as prognostic tests that can point to an agent in the current arsenal of chemotherapies to which a particular patient is most likely to respond. Cancer genomics has also helped to directly shape the development and use of some of the newest treatments.

The drug Gleevec, for example, was designed to inhibit an enzyme produced by a mutant fused version of two genes, called *BCR-ABL*, that causes chronic myelogenous leukemia. Gleevec is proving dramatically effective against that disease and showing value in the treatment of more genetically complex malignancies, such as gastrointestinal stromal tumor and several other relatively rare cancers that involve similar enzymes. Herceptin, an agent that targets a cellular signal–receiving protein called *HER2*, is successful against breast cancers with an abnormal multiplication of the *HER2* gene that causes overproduction of the receptor protein.

Strategies for selecting treatments based on specific gene mutations in a patient's cancer are also being tested in studies of the drugs Iressa and Tarceva for lung cancer, as well as Avastin for lung, colon and other cancers. The performance of these new gene-based diagnostics, prognostics and therapeutics is certainly good news, although the list of such interventions remains far shorter than it would be if researchers in academia and the private sector had ready access to the entire atlas of genomic changes that occur in cancer.

Recent studies illustrate both the potential power of large-scale genomics applied to the discovery of cancer genes and the tremendous undertaking a comprehensive cancer genome atlas will be. In 2006 Johns Hopkins University researchers sequenced about 13,000 genes in tumor tissues taken from 11

THE AUTHORS

FRANCIS S. COLLINS and *ANNA D. BARKER* are leaders of The Cancer Genome Atlas initiative in their positions as, respectively, director of the National Human Genome Research Institute and deputy director for Advanced Technologies and Strategic Partnerships of the National Cancer Institute. Collins led the Human Genome Project to its completion of the human DNA sequence, and Barker has headed drug development and biotechnology research efforts in the public and private sectors, with a particular focus on fighting cancer.

STEVE GSCHMEISSNER *Photo Researchers, Inc.*

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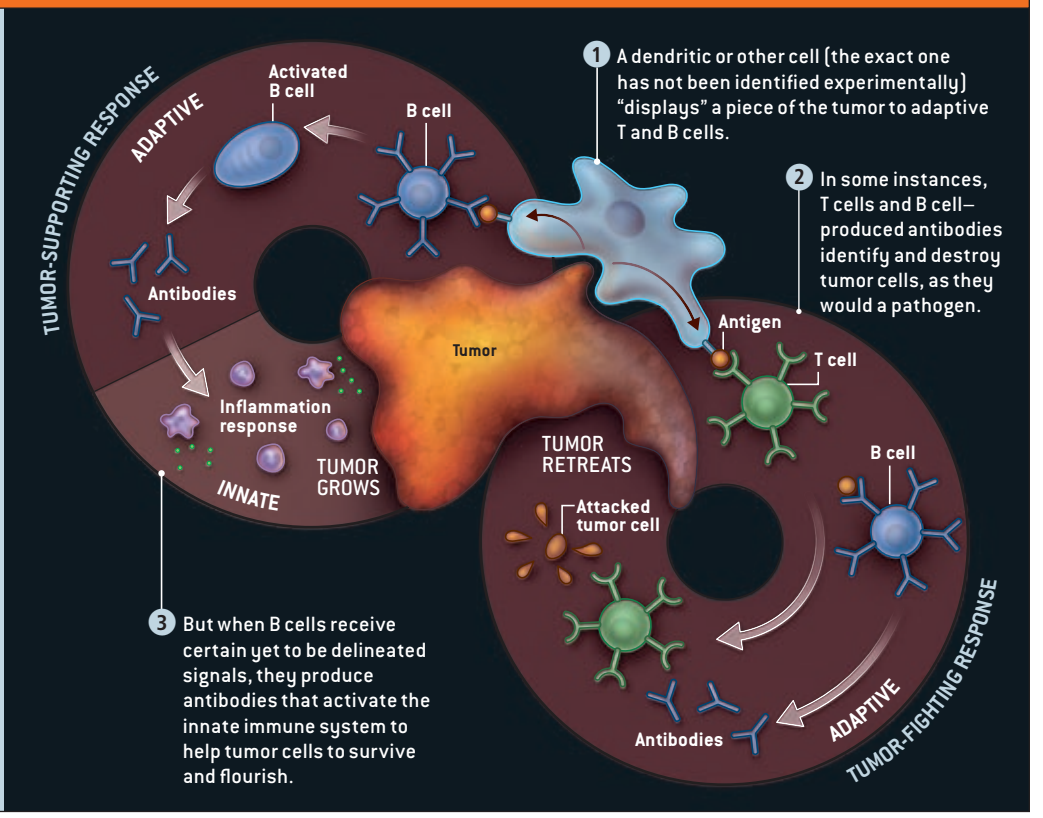
THE IMMUNE PARADOX

Two arms of the immune system—the innate and the adaptive—are exquisitely well adapted for fighting pathogens, but their role in combating cancer is decidedly more paradoxical. The innate system furnishes an initial inflammatory response to a microbial insult by attacking any invading pathogen indiscriminately, whereas adaptive immunity furnishes a delayed response that homes in on a particular pathogen. In cancer, both systems may sometimes attack tumor cells. But a tumor protects itself by recruiting the innate system to enhance its development.


TUMOR-SUPPORTING RESPONSE
ADAPTIVE
Activated B cell
B cell
Antibodies
Inflammation response
INNATE
TUMOR GROWS
Tumor
1 A dendritic or other cell (the exact one has not been identified experimentally) "displays" a piece of the tumor to adaptive T and B cells.
2 In some instances, T cells and B cell–produced antibodies identify and destroy tumor cells, as they would a pathogen.
Antigen
T cell
B cell
TUMOR RETREATS
Attacked tumor cell
Antibodies
ADAPTIVE
TUMOR-FIGHTING RESPONSE
3 But when B cells receive certain yet to be delineated signals, they produce antibodies that activate the innate immune system to help tumor cells to survive and flourish.

Genetic damage is the match that lights the fire of malignancy, and inflammation is the fuel that feeds the flames.

come factories for cytokines and growth factors that nurture tumor development.

Turning the macrophages into traitors begins when tumor cells send out help signals that attract cells that become macrophages once they reach the tumors. Inside the tumors, proliferating cells grow so quickly that they begin to die for lack of oxygen. A combination of hypoxia and messages from the tumor cells initiates a process whereby the newly arrived macrophages assume their bad-boy identity as tumor promoters. Cancer biologists give the name tumor-associated macrophages to these mutineers that congregate in and around the tumor.

Biologists have now been able to follow the inflammation link down to the level of individual signaling molecules, providing harder evidence for a connection to carcinogenesis. For example, nuclear factor-kappa B (NF-κB) is a complex of proteins that acts as a master switch for turning inflammation genes on and for controlling cell death. As biological pathways go, NF-κB's is world-famous, having been discovered and patented for use in drug development by scientific stars that include Nobelists David Baltimore and Phillip A. Sharp and having subsequently become the object of multimillion-dollar patent litigation.

In 2004 Yinon Ben-Neriah and Eli Pikarsky of the Hebrew University of Jerusalem and their colleagues reported that mice engineered to develop hepatitis (which can cause liver cancer) contracted precancerous lesions that did not progress to full malignancy when NF-κB was curtailed through a genetic alteration or when the proinflammatory TNF signaling molecule was shut off. In the latter group, a neutralizing antibody blocked TNF and prevented it from binding to a receptor on the premalignant liver cells; loss of the receptor prevented the TNF from triggering a molecular cascade that turns on the NF-κB master switch. Blocking NF-κB prompted the precancerous liver cells to initiate apoptosis, or programmed cell death. In a related finding that year, Michael Karin and his collaborators at the University of California, San Diego, found that inhibiting NF-κB in mice engineered to develop colitis, which can lead to colon cancer, also promoted apoptosis. Shutting down the pathway in inflammatory cells, such as macrophages, deterred tumor development as well.

So far the clearest evidence of a link between cancer and inflammation is the data demonstrating that inflammation encourages the conversion of precancerous tissue to full malignancy for many cancers. But the biolog-

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ical response may also be involved in initiating the disease and in advancing metastasis. Infections with *Helicobacter pylori* bacteria induce inflammation that greatly increases the risk of gastric cancer, and the hepatitis C virus can bring on liver cancer, to name just two malignancies. Pathogens may also generate free radicals, which can damage DNA. But although inflammation may be involved from the outset, few studies have shown yet that an inflammatory condition actually alters DNA to provide the initiating spark.

The case for a role in metastasis is stronger—and recent studies lend credence to this hypothesis. Karin's group reported in the April 5, 2007, *Nature* that inflammation, not genetic changes in cancer cells, spurs metastasis in mice engineered to acquire prostate cancer. The research suggests that a cytokine produced by inflammatory cells near a prostate tumor induces cancer cells to decrease production of a protein that blocks metastasis. This result, Karin notes, may explain the puzzling observation that cutting into tumors, such as for a prostate biopsy, sometimes seems to encourage metastasis. If he is correct, the inflammation generated by the intervention could be at fault. Around the same time, Pollard's group reported in *Cancer Research* on a study in mice that observed that macrophages accompany breast tumor cells in their migration toward blood vessels that then transport them to remote sites.

The innate immune system has received the most attention in explorations of how inflammation might cause cancer. As with in-

Immune System as Cancer Fighter

Tumors waylay the immune system to promote their own growth and survival. But the opposite also holds. The antibodies and killer T cells of the adaptive immune system can, at times, target and destroy cancer cells. Drug companies and scientists have tried to turn this knowledge into new therapies, with mixed results.

Among the most successful new biotechnology drugs are monoclonal antibodies—identical antibodies that are capable of attacking a cancer antigen, a molecular fragment found on the surface of a cancer cell. Monoclonals are generally a "passive" immunotherapy because they are produced in cell culture or in mice and injected into patients instead of relying on the patient's own immune system to produce antibodies.

In contrast, cancer vaccines—the object of a frustrating decades-long quest—are "active" therapies. A patient receives an injection of an antigen, usually along with another helper molecule, an adjuvant, that precipitates an immune response.

Cancer antigens are more difficult to identify than those for pathogens because cancer cells are mutant forms of the body's own cells. The adaptive immune system often does not see them as foreign—and a tumor can trick the body into turning off any immune response that does arise.

After hundreds of previous trials, and the absence of virtually any evidence from them that vaccines cause tumors to regress, the concept of the cancer vaccine has garnered its share of critics. "When you give cancer vaccines, sometimes you raise T cells against the vaccines, but they're just not powerful enough to keep the cancer from growing," notes Steven A. Rosenberg, chief of surgery at the National Cancer Institute. Rosenberg and his co-workers have pursued a different approach, adoptive cell transfer, in which T cells that target tumors are selected from white blood cells removed from the body. They are then multiplied in the laboratory and reinfused into patients whose immune systems have been chemically suppressed. In a 2005 study in the *Journal of Clinical Oncology,* about half of 35 melanoma patients saw their metastatic tumors regress at least partially.

In recent years, the picture for cancer vaccines has brightened somewhat. Early positive reports were presented at the American Association for Cancer Research in April 2007 for vaccines for breast, prostate, and head and neck cancers. But more bad news came the next month: the Food and Drug Administration delayed approval of what would have been the first U.S. therapeutic cancer vaccine, formulated by Seattle-based Dendreon for prostate cancer.

Marshaling the patient's own immune system to fight cancer may still be possible. But meeting that goal may well depend on deepening the growing understanding of how the immune system serves as a two-edged sword that can either foster or block cancer progression. —*G.S.*

COMMON CAUSE

Chronic inflammation contributes to many diseases, not just cancer.

HEART DISEASE

Macrophages, stars of innate immunity, are key players. They ingest "bad" cholesterol (low-density lipoproteins), and then the cells are encased in a fibrous cap that forms arterial plaque, which can break off and create a clot that blocks an artery, leading to a heart attack.

DIABETES

When exposed to the metabolic stress that occurs from being obese, both innate immune cells and fat cells (adipocytes) manufacture signaling molecules called cytokines, such as tumor necrosis factor. These molecules interfere with the normal function of insulin and can lead to diabetes.

ALZHEIMER'S DISEASE

Microglial cells, the neural equivalent of macrophages, express cytokines and cell-damaging free radicals, while interacting with the beta-amyloid proteins that build up in the plaques that are characteristic of the disease. The resulting inflammation can damage neurons.

DEPRESSION AND SCHIZOPHRENIA

High levels of inflammatory molecules—interleukin-6 and C-reactive protein—have been found in depressed patients. Some evidence even suggests that elevated cytokines correlate with schizophrenia.

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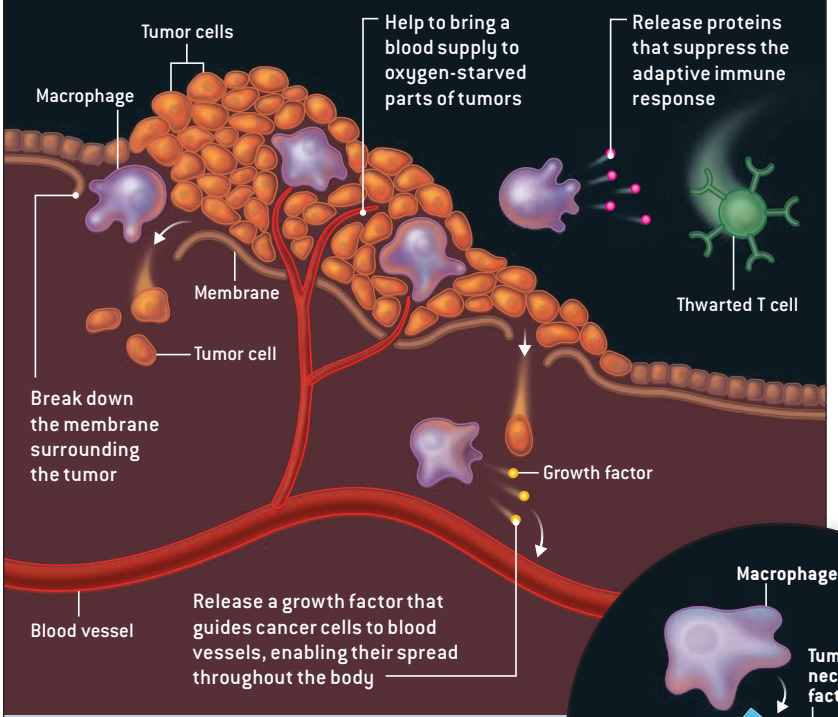
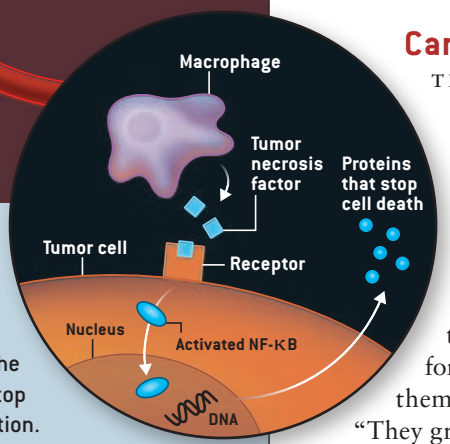
THE BIG EATERS

Macrophages, also known as big eaters, are inflammatory cells that coordinate many critical steps in cancer development, from nurturing the tumor to helping its cells spread to distant sites.


Tumor cells
Macrophage
Help to bring a blood supply to oxygen-starved parts of tumors
Release proteins that suppress the adaptive immune response
Membrane
Thwarted T cell
Tumor cell
Break down the membrane surrounding the tumor
Growth factor
Blood vessel
Release a growth factor that guides cancer cells to blood vessels, enabling their spread throughout the body



A MASTER SWITCH ▶

Macrophages produce inflammatory compounds, such as tumor necrosis factor, which can activate a gene switch, a complex of proteins called nuclear factor-kappa B (NF-κB), in a tumor cell. NF-κB enters the cell nucleus and turns on production of proteins that stop cell death and promote inflammation and cell proliferation.

nate immunity, the adaptive immune system—the T cells and antibodies produced by B cells that target specific molecules on invading cells—contributes to pathology or may also fight against it. For decades, immunotherapies designed to enhance T cell responses against cancer have been explored, though often with disappointing results [*see box on preceding page*].

Furthermore, an emerging picture has begun to reveal an intricate cross talk between innate and adaptive immune cells that may participate in the promotion of malignant disease. Researchers working on cancer vaccines may need to take account of these interactions in designing their treatments if they are ever to prove effective. One study showed that ovarian tumors produce a signaling molecule that serves to attract regulatory T cells, a subclass of adaptive immune cells responsible for quieting other T cells.

Meanwhile Coussens and her colleagues at U.C.S.F. found in a 2005 study, published in *Cancer Cell*, that the removal of antibody-making B cells from mice engineered to be prone to skin cancer prevented the tissue changes and angiogenesis that are prerequisites for disease progression. In their normal role as pathogen fighters, B cell–produced antibodies circulate through the bloodstream and mark viruses and bacteria for destruction by innate immune cells. In response to a signal from precancerous tissue, however, the antibodies induce the innate system to collaborate in cancer development. An open research question is how this process starts. One possibility suggests that a cancer cell may send a message to innate immune cells, perhaps dendritic cells, that then activate B cells. Signaling may involve toll-like receptors, which have emerged as prominent intermediaries in innate immune messaging.

Cancer Blockers

THE RECOGNITION that cancer is more like an organ than just a clump of cells with DNA mutations in cell nuclei may also explain why some of the previous approaches to chemotherapy have met with limited success. "People have taken cells and then transformed them in culture and stuck them into animals," Pollard says. "They grow as little balls. They do certain things there. But they are not complex tissues, whereas a naturally occurring tumor is a very complex tissue."

Instead of just killing cancer cells—the goal of current drug therapies and radiation—new approaches may supplement existing drugs by slowing inflammation. Without the involvement of macrophages and other innate cells, the premalignant tissue would remain in check. Cancer could, in essence, become a chronic disease akin to rheumatoid arthritis, another inflammatory condition. "Keep in mind almost no one dies of primary cancer," says Raymond DuBois, provost of the University of Texas M. D. Anderson Cancer Center and a researcher of anti-inflammatory agents for cancer. "A patient almost always dies from a metastasis."

A pharmaceutical against chronic inflammation represents a more alluring proposition than massacring malignant cells (and, un-

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avoidably, healthy ones), a consequence of existing chemotherapies. Taken alone, such an agent might be benign enough to use every day as a preventive for high-risk patients. Epidemiological and clinical studies have shown some promise for the use of nonsteroidal anti-inflammatory drugs (NSAIDs) such as aspirin to stave off the onset of some solid tumors. Investigations continue on more selective blocking of the production of prostaglandins, the regulatory molecules that are curtailed by NSAIDs. In particular, drugs that inhibit production of prostaglandin E_2 may curb inflammation and tumor growth, while avoiding the cardiovascular side effects of drugs such as Vioxx and the gastrointestinal problems of the earlier class of NSAIDs. The anti-inflammatory effects of the ubiquitous statins used to lower cholesterol are also being contemplated.

Some treatment options already exist. The drug Avastin inhibits production of the angiogenesis-promoting VEGF, although oncologists must contend with other molecules in the tumor microenvironment that promote blood vessel growth. Drugs developed for more familiar inflammatory diseases may also fight cancer—and these medicines might be combined into HIV-like drug cocktails that also include angiogenesis inhibitors and cell-killing agents.

Inhibitors of TNF have received approval for treatment of rheumatoid arthritis, Crohn's disease and other disorders and are now in clinical trials for both solid tumors and blood cancers. The drug Rituxan, a monoclonal antibody that represses B cells in rheumatoid arthritis and B cell lymphoma, might prevent the inflammatory response that fuels formation of solid tumors. Other cytokines and related molecules (IL-6, IL-8 and CCL2, among others) are also potential targets, as is NF-κB.

Some existing compounds, including NSAIDs and even one found in the spice turmeric, exert at least some of their effects by inhibiting NF-κB. But major pharmaceutical laboratories are investigating highly selective inhibitors of this molecular linchpin, many of them targeted at the enzymes (such as I-κB kinase) that regulate NF-κB activity.

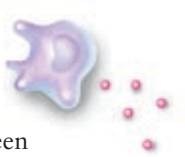
A Chemical Trojan

ONE GROUP IS contemplating a radically ambitious treatment, a molecular Trojan horse of sorts. Claire E. Lewis and Munitta Muthana of the University of Sheffield in England and their colleagues have designed a drug delivery scheme that takes advantage of the natural attraction of macrophages to the oxygen-starved areas in tumors. They have engineered macrophages to deliver a therapeutic virus to hypoxic tumor regions, which respond poorly to conventional treatments such as chemotherapy and radiation because of an insufficient blood supply. Once the macrophages arrive in a tumor (grown in culture so far), each one releases thousands of copies of the virus, which then infect the cancer cells, after which a protein in those cells activates the therapeutic gene in each virus. This action then directs synthesis of a cell-killing toxin. "The macrophage is migrating into a site and doing what we want it to do rather than driving tumor development in a normal way," Lewis says.

The exact outlines of an anti-inflammatory strategy against cancer have yet to be elucidated. Tweaking immune cells that form a defensive barrier against pathogens bears its own risks. "It's a very complicated issue," DuBois notes. "If you magically shut down the immune system, you will have problems with opportunistic infections, just like with AIDS." Use of TNF blockers in other inflammatory disorders has been linked to tuberculosis and other infections, even potentially lymphoma. Moreover, inhibiting the NF-κB pathway can paradoxically promote cancer in some instances. Constraining NF-κB can at times lead to tissue damage and a process of abnormal regeneration of that tissue that can foster cancer.

Still, it seems likely that a new generation of anti-inflammatory agents will join the chemotherapeutic arsenal. Chronic diseases—and their underlying inflammatory conditions—are hallmarks of an aging population. "We're all a little bit overinflamed," Pollard observes. Treating the smoldering embers that surround the tumor rather than just mutant cells could make cancer a disease we can live with. SA

Instead of killing cancer cells—the goal of current drug therapies and radiation—new chemotherapies may supplement existing drugs by turning down inflammation.



Gary Stix is senior writer for Scientific American.

MORE TO EXPLORE

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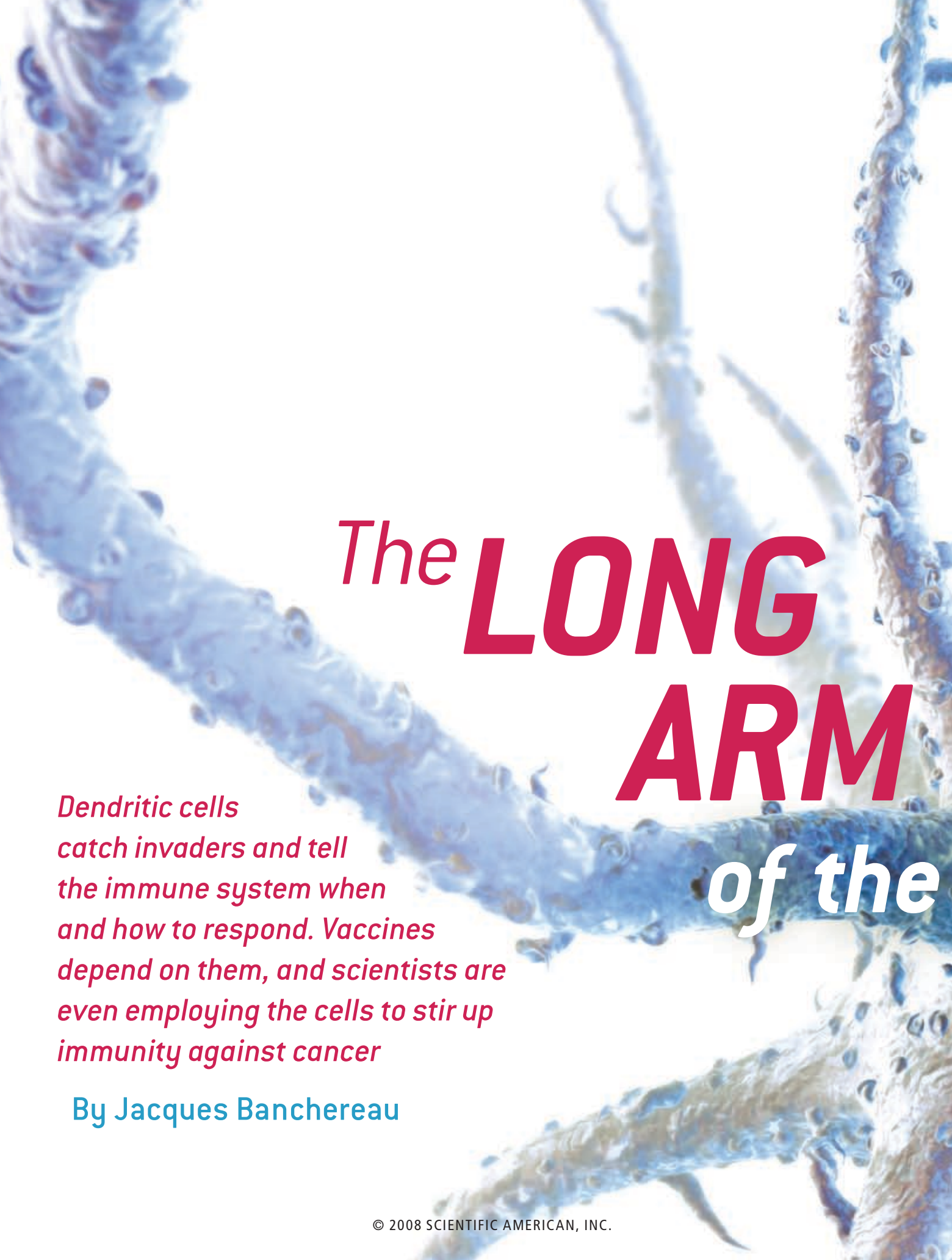

The LONG ARM of the

Dendritic cells catch invaders and tell the immune system when and how to respond. Vaccines depend on them, and scientists are even employing the cells to stir up immunity against cancer

By Jacques Banchereau

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Immune System

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They lie buried—their long, tentaclelike arms outstretched—in all the tissues of our bodies that interact with the environment. In the lining of our nose and lungs, lest we inhale the influenza virus in a crowded subway car. In our gastrointestinal tract, to alert our immune system if we swallow a dose of salmonella bacteria. And most important, in our skin, where they lie in wait as stealthy sentinels should microbes breach the leathery fortress of our epidermis.

They are dendritic cells, a class of white blood cells that encompasses some of the least understood but most fascinating actors in the immune system. Over the past decade, researchers have begun to unravel the mysteries of how dendritic cells educate the immune system about what belongs in the body and what is foreign and potentially dangerous. Intriguingly, they have found that dendritic cells initiate and control the overall immune response. For instance, the cells are crucial for establishing immunological "memory," which is the basis of all vaccines. Indeed, physicians, including those at a number of biotechnology companies, are taking advantage of the role that dendritic cells play in immunization by "vaccinating" cancer patients with dendritic cells loaded with bits of their own tumors to activate their immune system against their cancer. Dendritic cells are also responsible for the phenomenon of immune tolerance, the process through which the immune system learns not to attack other components of the body.

But dendritic cells can have a dark side. The human immunodeficiency virus (HIV) hitches a ride inside dendritic cells to travel to lymph nodes, where it infects and wipes out helper T cells, causing AIDS. And those cells that become active at the wrong time might give rise to autoimmune disorders such as lupus. In these cases, shutting down the activity of dendritic cells could lead to new therapies.

Overview/*Dendritic Cells*

- Dendritic cells—named for their long arms, or dendrites—exist in many tissues, particularly the skin and mucous membranes. They reel in invaders, chop them into pieces called antigens and display the antigens on their surfaces.
- Antigen-bearing dendritic cells travel to lymph nodes or the spleen, where they interact with other cells of the immune system—including B cells, which make antibodies, and killer T cells, which attack microbes and infected cells.
- Cancer vaccines composed of dendritic cells bearing tumor antigens are now in clinical trials involving humans. Scientists are also hoping to turn off the activity of dendritic cells to combat autoimmune diseases such as lupus.

Rare and Precious

DENDRITIC CELLS are relatively scarce: they constitute only 0.2 percent of white blood cells in the blood and are present in even smaller proportions in tissues such as the skin. In part because of their rarity, their true function eluded scientists for nearly a century after they were first identified in 1868 by German anatomist Paul Langerhans, who mistook them for nerve endings in the skin.

In 1973 Ralph M. Steinman of the Rockefeller University rediscovered the cells in mouse spleens and recognized that they are part of the immune system. The cells were unusually potent in stimulating immunity in experimental animals. He renamed the cells "dendritic" because of their spiky arms, or dendrites, although the subset of dendritic cells that occur in the epidermis layer of the skin are still commonly called Langerhans cells. This groundbreaking research laid the foundation for all of the progress that we are seeing in dendritic cell science today. In 2007 Steinman received the Albert Lasker Basic Medical Research Award for his pioneering work in rediscovering and characterizing dendritic cells.

For almost 20 years after the cells' rediscovery, researchers had to go through a painstakingly slow process to isolate them from fresh tissue for study.

ILLUSTRATION BY JEFF JOHNSON *Hybrid Medical Animation [preceding pages]*

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But in 1992, when I was at the Schering-Plough Laboratory for Immunology Research in Dardilly, France, my co-workers and I devised methods for growing large amounts of human dendritic cells from bone marrow stem cells in culture dishes in the laboratory. At roughly the same time, Steinman—in collaboration with Kayo Inaba of Kyoto University in Japan and her colleagues—reported that he had invented a technique for culturing dendritic cells from mice.

In 1994 researchers led by Antonio Lanzavecchia, now at the Institute for Research in Biomedicine in Bellinzona, Switzerland, and Gerold Schuler, now at the University of Erlangen-Nuremberg in Germany, found a way to grow the cells from white blood cells called monocytes. Scientists now know that monocytes can be prompted to become either dendritic cells, which turn the immune system on and off, or macrophages, cells that crawl through the body scavenging dead cells and microbes.

The ability to culture dendritic cells offered scientists the opportunity to investigate them in depth for the first time. Some of the initial discoveries expanded the tenuous understanding of how dendritic cells function.

There are several subsets of dendritic cells, which arise from precursors that circulate in the blood and then take up residence in immature form in the skin, mucous membranes, and organs such as the lungs and spleen. Immature dendritic cells are endowed with a wealth of mechanisms for capturing invading microbes: they reel in invaders using suction cup–like receptors on their surfaces, they take microscopic sips of the fluid surrounding them, and they suck in viruses or bacteria by engulfing them in sacks known as vacuoles. Yong-Jun Liu, a former colleague of mine from Schering-Plough who is now at the University of Texas M. D. Anderson Cancer Center, has found that some immature dendritic cells can also zap viruses immediately by secreting a substance called interferon-alpha.

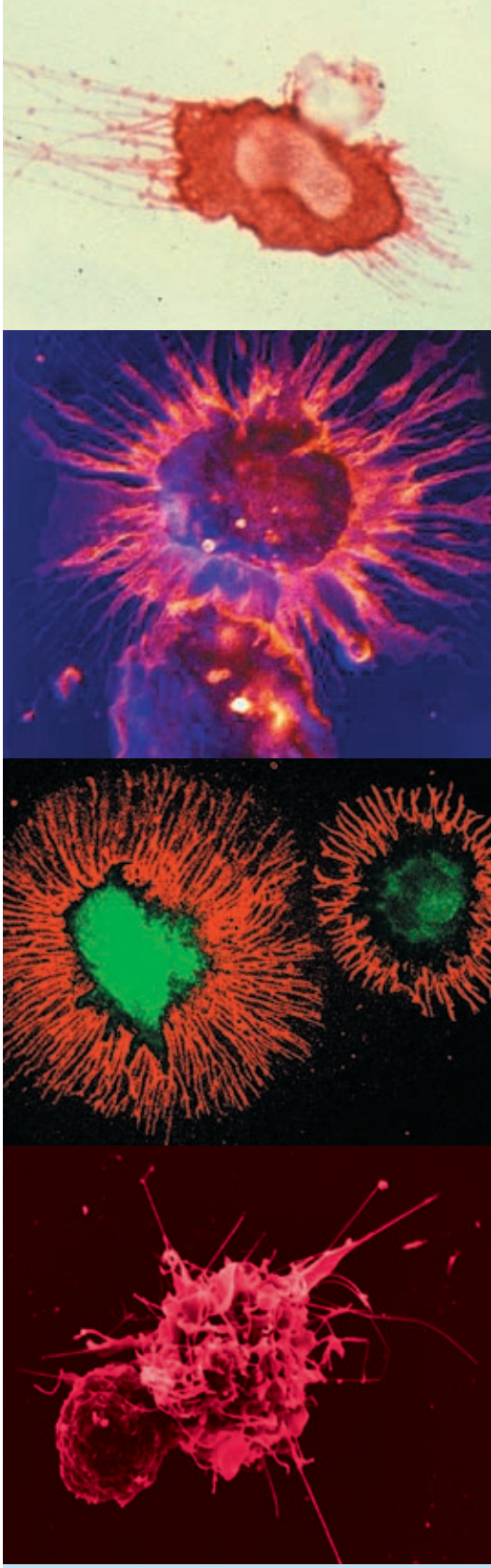
Once they devour foreign objects, the immature cells chop them into fragments (antigens) that can be recognized by the rest of the immune system [*see box on next two pages*]. The cells use pitchfork-shaped molecules termed the major histocompatibility complex (MHC) to display the antigens on their surfaces. The antigens fit between the tines of the MHC, which comes in two types, class I and class II. The two types vary in shape and in the ways they acquire their antigen cargo while residing inside cells.

Dendritic cells are very efficient at capturing and presenting antigens: they can pick up antigens that occur in only minute concentrations. As they process antigens for presentation, they travel to the spleen through the blood or to lymph nodes through a clear fluid known as lymph. Once at their destinations, the cells complete their maturation and present their antigen-laden MHC molecules to naive helper T cells, those that have never encountered antigens before. Dendritic cells are the only cells that can educate naive helper T cells to recognize an antigen as foreign or dangerous. This unique ability appears to derive from costimulatory molecules on their surfaces that can bind to corresponding receptors on the T cells.

Once educated, the helper T cells go on to prompt so-called B cells to produce antibodies that bind to and inactivate the antigen. The dendritic cells and helper cells also activate killer T cells, which can destroy cells infected by microbes. Some of the cells that have been educated by dendritic cells become "memory" cells that remain in the body for years—perhaps decades—to combat the invader in case it ever returns.

Whether the body responds with antibodies or killer cells seems to be determined in part by which subset of dendritic cell conveys the message and which of two types of immune-stimulating substances, called cytokines, the dendritic cells prompt the helper T cells to make. In the case of parasites or some bacterial invaders, type 2 cytokines are best because they arm the immune system with antibodies; type 1 cytokines are better at mustering killer cells to attack cells infected by other kinds of bacteria or by viruses.

If a dendritic cell prompts the wrong



SPIKY ARMS are common to mature dendritic cells from humans (*top* and *top middle*), mice (*bottom middle*) and rats (*bottom*). The rat dendritic cell is interacting with what is probably a helper T cell. Through such interactions, dendritic cells teach the immune system what it should attack. Cells matured in the laboratory, such as the one at the top middle, are being used in cancer vaccines.

JACQUES BANCHEREAU (*top* and *top middle*); OLIVIA GRESSER *Marseilles-Luminy Center of Immunology INSERM/CNRS* (*bottom middle*); WILLIAM E. BOWERS, STEVE HALEY AND RICHARD HUNT *University of South Carolina* (*bottom*)

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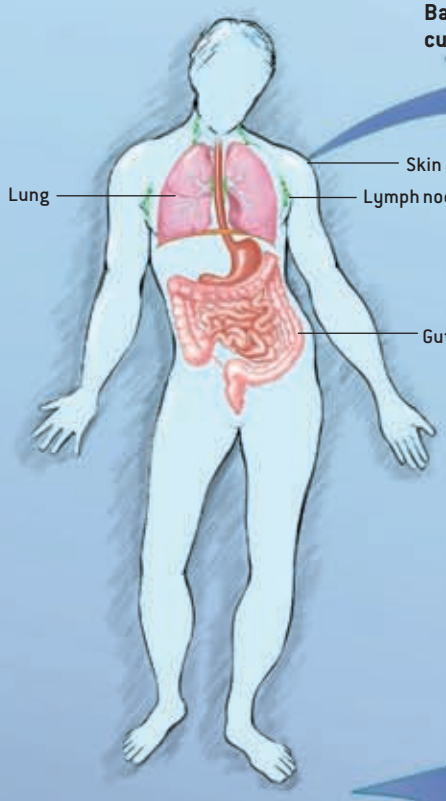
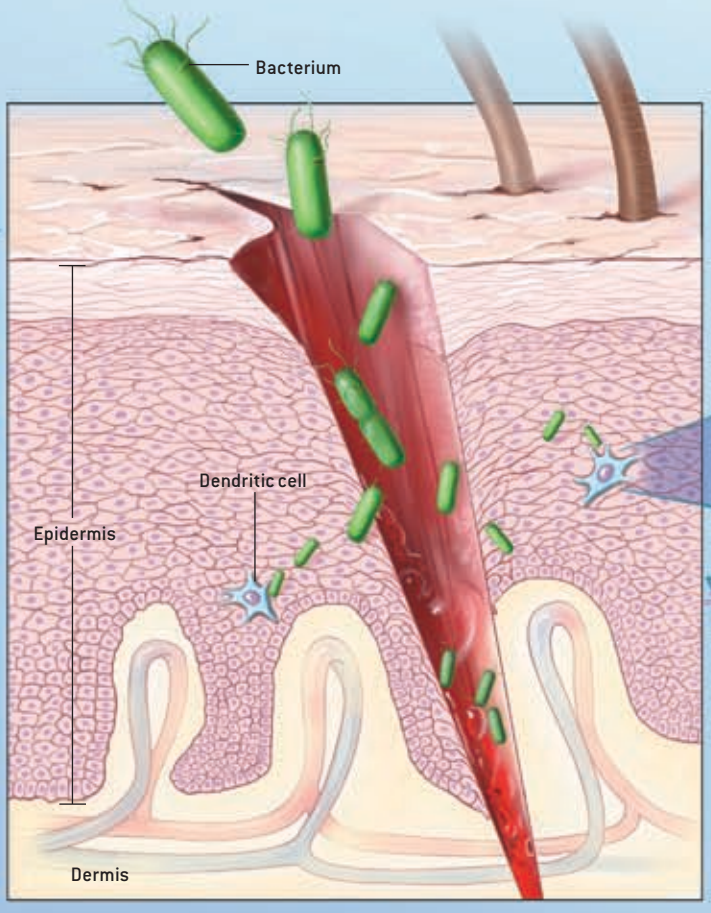
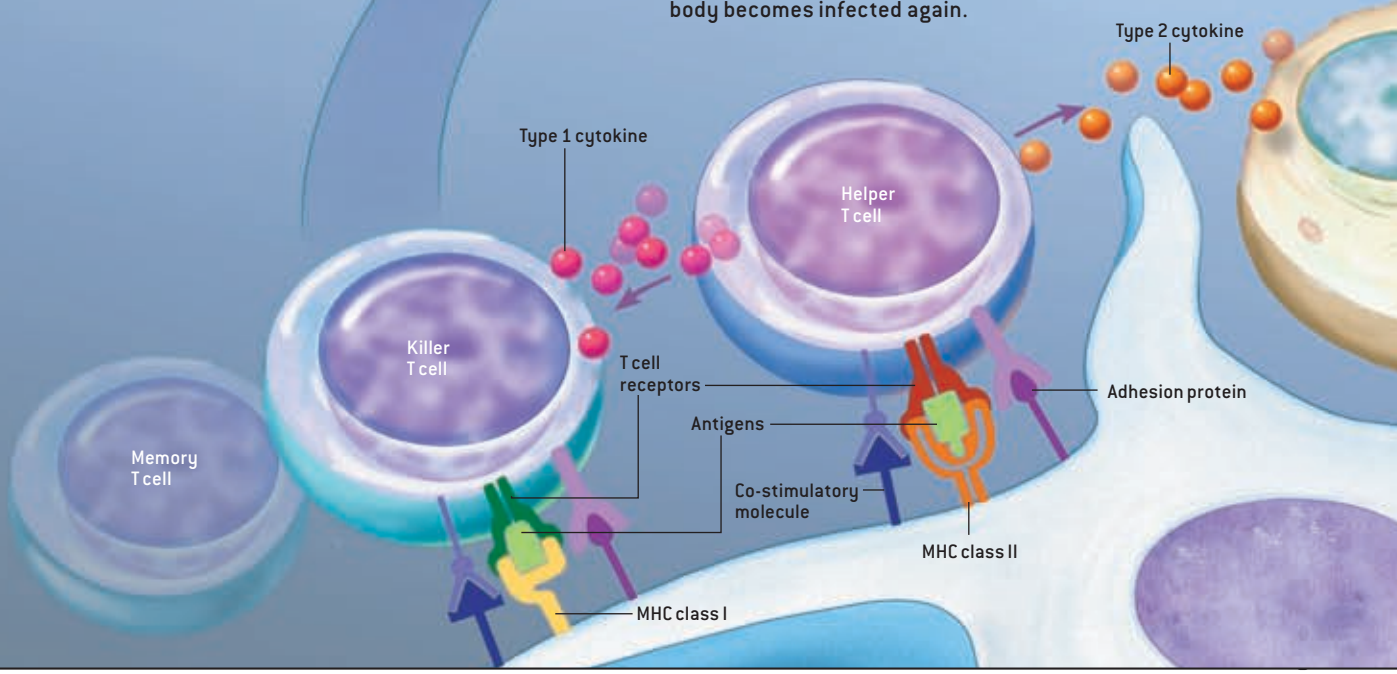
DENDRITIC CELLS AND INFECTION

Present in the lungs, skin, gut and lymph nodes, dendritic cells orchestrate the immune response against invaders (here, bacteria entering a cut in the skin).





Dendritic cells bind to helper T cells, killer T cells and—perhaps—B cells. The binding prompts the helper T cells to make substances called cytokines that stimulate killer T cells and cause B cells to begin making antibodies. The antibodies and killer T cells migrate to the cut to fight the infection. Memory cells persist in case the body becomes infected again.



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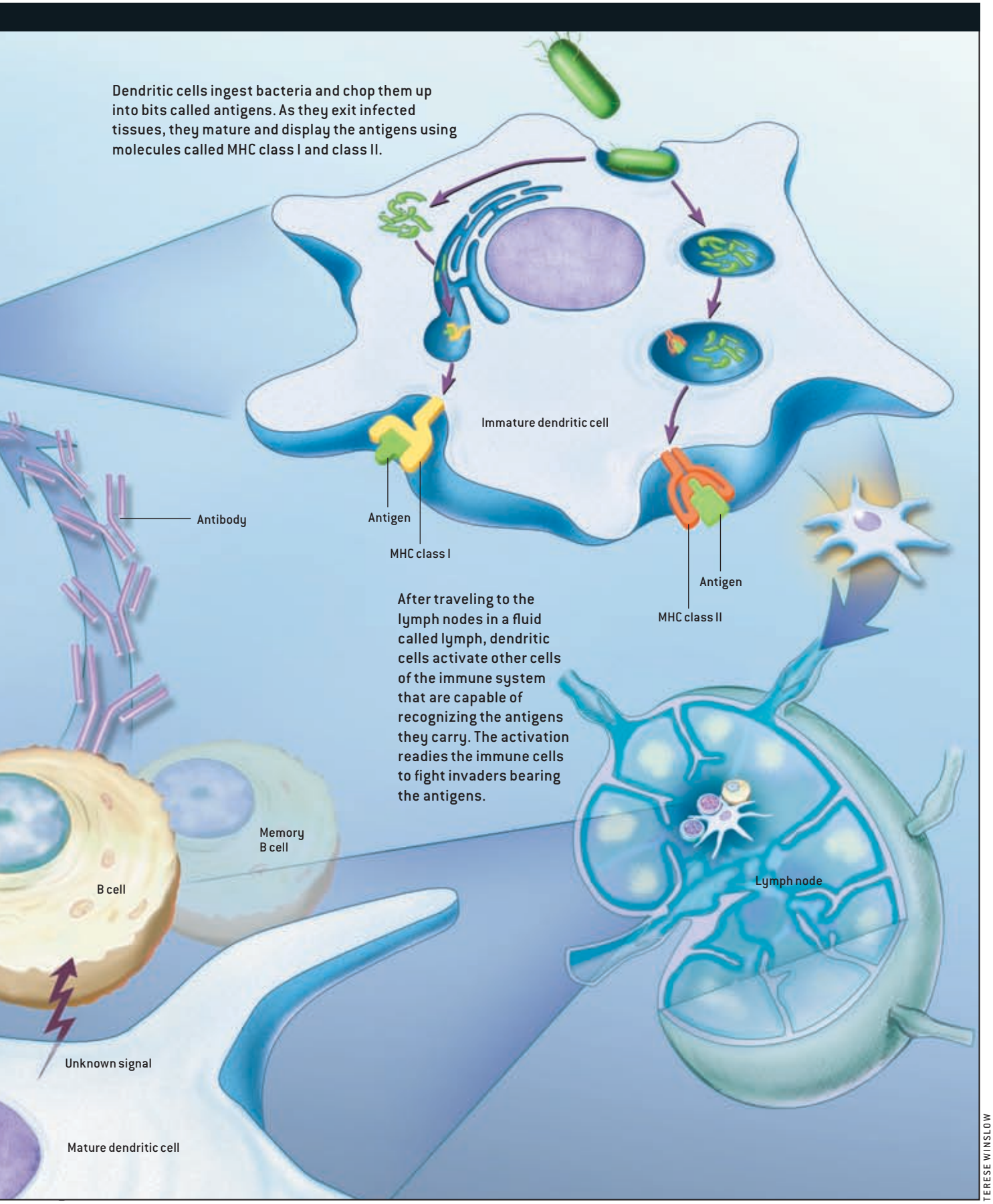
Dendritic cells ingest bacteria and chop them up into bits called antigens. As they exit infected tissues, they mature and display the antigens using molecules called MHC class I and class II.
Immature dendritic cell
Antibody
Antigen
MHC class I
Antigen
MHC class II
After traveling to the lymph nodes in a fluid called lymph, dendritic cells activate other cells of the immune system that are capable of recognizing the antigens they carry. The activation readies the immune cells to fight invaders bearing the antigens.
Memory B cell
B cell
Lymph node
Unknown signal
Mature dendritic cell

TERESE WINSLOW

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type of cytokine, the body can mount the wrong offense. Generating the appropriate kind of immune response can be a matter of life or death: when exposed to the bacterium that causes leprosy, people who mount a type 1 response develop a mild, tuberculoid form of the disease, whereas those who have a type 2 response can end up with the potentially fatal lepromatous form.

Cancer Killers

ACTIVATING NAIVE helper T cells is the basis of vaccines for everything from pneumonia to tetanus to influenza. Scientists are now turning the new knowledge of the role that dendritic cells play in immunity against microbes and their toxins into a strategy to fight cancer.

Cancer cells are abnormal and as such are thought to generate molecules that healthy cells do not. If researchers could devise drugs or vaccines that exclusively targeted those aberrant molecules, they could combat cancer more effectively while leaving normal cells and tissues alone—thereby eliminating some of the pernicious side effects of chemotherapy and radiation, such as hair loss, nausea and weakening of the immune system caused by destruction of the bone marrow.

Antigens that occur only on cancerous cells have been hard to find, but researchers have succeeded in isolating several of them, most notably from the skin cancer melanoma. In the early 1990s Thierry Boon of the Ludwig Cancer Institute in Brussels, Steven A. Rosenberg of the National Cancer Institute and their colleagues independently identified melanoma-specific antigens that are currently being targeted in a variety of clinical trials involving humans.

Such trials generally employ vaccines made of dendritic cell precursors that have been isolated from cancer patients and grown in the laboratory together with tumor antigens. During this process, the dendritic cells pick up the antigens, chop them up and present them on their surfaces. When injected back into the patients, the antigen-loaded dendritic cells are expected to ramp up patients' immune response against their own tumors.

Various researchers—including our own group as well as scientists at several biotechnology companies—are testing this approach against cancers as diverse as melanoma, B cell lymphoma, and tumors of the prostate and colon. There have been glimmers of success. In September 2001, for instance, my co-workers and I, in collaboration with Steinman's group, reported that 16 of 18 patients with advanced melanoma to whom we gave injections of dendritic cells loaded with melanoma antigens showed signs in laboratory tests of an enhanced immune response to their cancer. What is more, tumor growth was slowed in the nine patients who mounted responses against more than two of the antigens.

Scientists are now working to refine the approach and test it on larger numbers of patients. So far cancer vaccines based on dendritic cells have been tested only in patients with advanced cancer. Although researchers believe that patients with earlier-stage cancers may respond better to the therapy—their immune systems have not yet tried and failed to eradicate their tumor—several potential problems must first be considered.

Some researchers fear that such vaccines might induce patients' immune systems to attack healthy tissue by mistake. For instance, vitiligo—white patches on the skin caused by the destruction of normal pigment-producing melanocytes—has been observed in melanoma patients who have received the earliest antimelanoma vaccines. (No major adverse events have been reported, however, in more than 1,000 vaccinated patients.) Conversely, the tumors might mutate to "escape" the immune onslaught engendered by a dendritic cell vaccine. Tumor cells could accomplish this evasion by no longer making the antigens the vaccine was designed to stimulate the immune system against. This problem is not unique to dendritic cells, though: the same phenomenon can occur with traditional cancer therapies.

In addition, tailoring a dendritic cell vaccine to fight a particular patient's tumors might not be economically feasible. But many scientists are working to circumvent the costly and time-consuming steps of isolating cells from patients and manipulating them in the laboratory for reinjection.

One approach involves prompting dendritic cell precursors already present in a person's body to divide and start orchestrating an immune response against their tumors. While at Immunex in Seattle, David H. Lynch, now at Bainbridge Biopharma Consulting in Bainbridge Island, Wash., and his co-workers discovered a cytokine that causes mice to make more dendritic cells, which eventually induce the animals to reject grafted tumors. Other scientists, including Drew M. Pardoll of Johns Hopkins University, have observed that tumor cells that have been genetically engineered to secrete large amounts of cytokines that activate dendritic cells have the most potential as cancer vaccines.

Another approach, pioneered by Steinman and his Rockefeller colleague Michel C. Nussenzweig, is to selectively target antigens by coupling them to monoclonal antibodies that bind to receptors on the surface of dendritic cells. These receptors need to allow internalization of the antigens and their processing for presentation on both MHC class I and class II antigens. Several such molecules are now under intense scrutiny. Studies in mice have shown that targeting the antigens in the absence of dendritic cell activation results in tolerance induction. In contrast, delivering the antigen together with dendritic cell activators induces immunity, which can be protective.

THE AUTHOR

JACQUES BANCHEREAU has directed the Baylor Institute for Immunology Research in Dallas since 1996. The institute aims to manipulate the human immune system to treat cancer as well as infectious and autoimmune diseases. Before 1996 Banchereau led the Schering-Plough Laboratory for Immunology Research in Dardilly, France. He obtained his Ph.D. in biochemistry from the University of Paris and holds many patents on immunological techniques.

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Shutting Immunity Down

IN THE MEANTIME, other scientists are looking at ways to turn off the activity of dendritic cells in instances where they exacerbate disease instead of fighting it. Usually, in a phenomenon known as central tolerance, an organ in the chest called the thymus gets rid of young T cells that happen to recognize the body's own components as foreign before they have a chance to circulate. Some inevitably slip through, however, so the body has a backup mechanism for restraining their activity.

But this mechanism, termed peripheral tolerance, appears to be broken in patients with autoimmune disorders such as rheumatoid arthritis, type 1 diabetes and systemic lupus erythematosus. In 2001 my colleagues and I reported that dendritic cells from the blood of people with lupus are unnaturally active. Cells from these patients release high amounts of interferon-alpha, an immune-stimulating protein that causes precursors to grow into mature dendritic cells while still in the bloodstream. The mature cells then ingest DNA, which is present in unusual amounts in the blood of people with lupus, and that in turn causes the individual's immune system to generate antibodies against his or her own DNA. These antibodies result in the life-threatening complications of lupus when they lodge in the kidneys or the walls of blood vessels.

Accordingly, we propose that blocking interferon-alpha might lead to a therapy for lupus by preventing dendritic cell activation. A similar strategy might prevent organ transplant recipients from rejecting their new tissues.

A new treatment for AIDS might also rest on a better understanding of dendritic cells. In 2000 Carl G. Figdor and Yvette van Kooyk, then at the University Medical Center St. Radboud in Nijmegen, the Netherlands, identified a subset of dendritic cells that makes DC-SIGN, a molecule that can bind to the outer coat of HIV. These cells pick up HIV as they regularly prowl the mucous membranes and deep tissues. When they travel to the lymph nodes, they unwittingly deliver the virus to a large concentration of T cells. Drugs that block the interaction between DC-SIGN and HIV might slow the progression of AIDS.

Other infectious diseases—including malaria, measles and cytomegalovirus—also manipulate dendritic cells for their own ends. Red blood cells that have been infected by malaria parasites, for instance, bind to dendritic cells and prevent them from maturing and alerting the immune system to the presence of the invaders. Several groups of researchers are now devising approaches to prevent such microbes from hijacking dendritic cells; some are even seeking to use supercharged dendritic cells to fight the infections.

As we learn more about the molecules that control dendritic cells, we will find ways to harness their therapeutic potential. The increasing number of scientists and pharmaceutical corporations working on dendritic cells portends that we will soon be able to maximize the biological power of these cells to treat and prevent the diseases that plague humankind. SA

MORE TO EXPLORE

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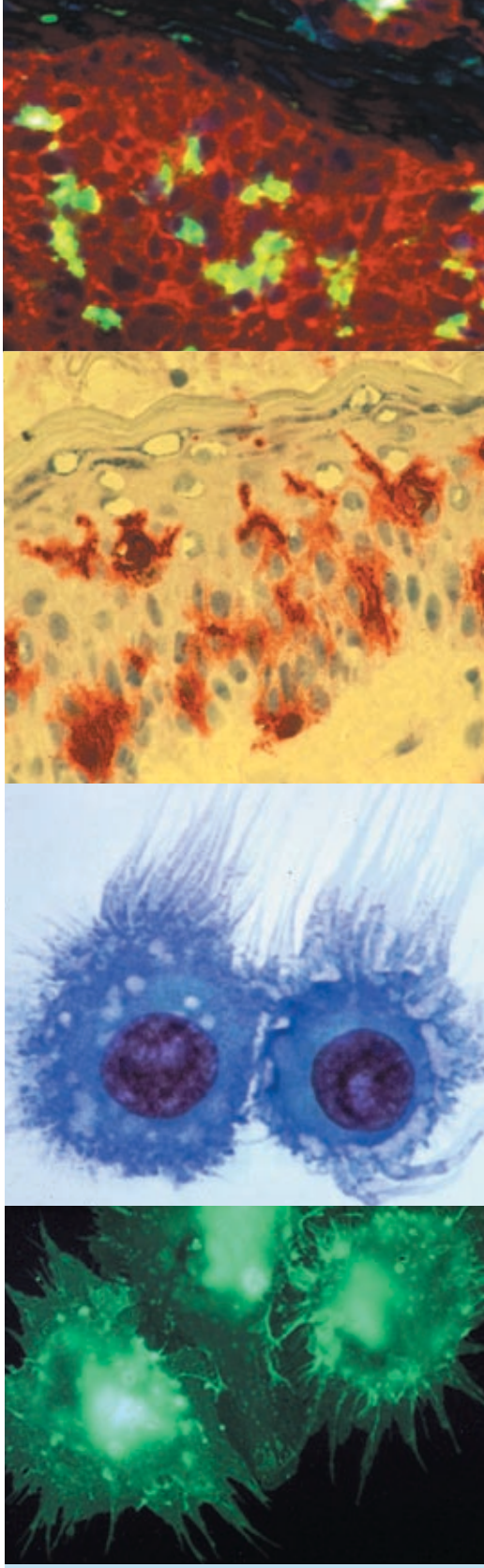
Dendritic Cells as Vectors for Therapy. Jacques Banchereau, Beatrice Schuler-Thurner, A. Karolina Palucka and Gerold Schuler in *Cell,* Vol. 106, No. 3, pages 271–274; August 10, 2001.

Taking Dendritic Cells into Medicine. Ralph M. Steinman and Jacques Banchereau in *Nature,* Vol. 449, pages 419–426; September 27, 2007.

Dendritic Cell Subsets in Health and Diseases. H. Ueno, E. Klechevsky, R. Morita, C. Aspord, T. Cao, T. Matsui, T. Di Pucchio, J. Connolly, J. W. Fay, V. Pascual, A. K. Palucka and J. Banchereau in *Immunological Reviews,* Vol. 219, No. 1, pages 118–142; October 2007.

Background information on the immune system and on experimental cancer therapies such as those using dendritic cells can be found on the American Cancer Society's Web site: **www.cancer.org**



IMMATURE DENDRITIC CELLS can be stained to show up green in breast cancer tissue (*top*) or red in normal skin (*top middle*). As the cells mature, they make proteins that allow them to stick to one another (*bottom middle*). They also produce forklike receptors (*green dots, bottom*), which they use to show bits of invaders to other immune cells.

JACQUES BANCHEREAU (*top, top middle* and *bottom middle*); MATTHEW L. ALBERT *Rockefeller University* (*bottom*)

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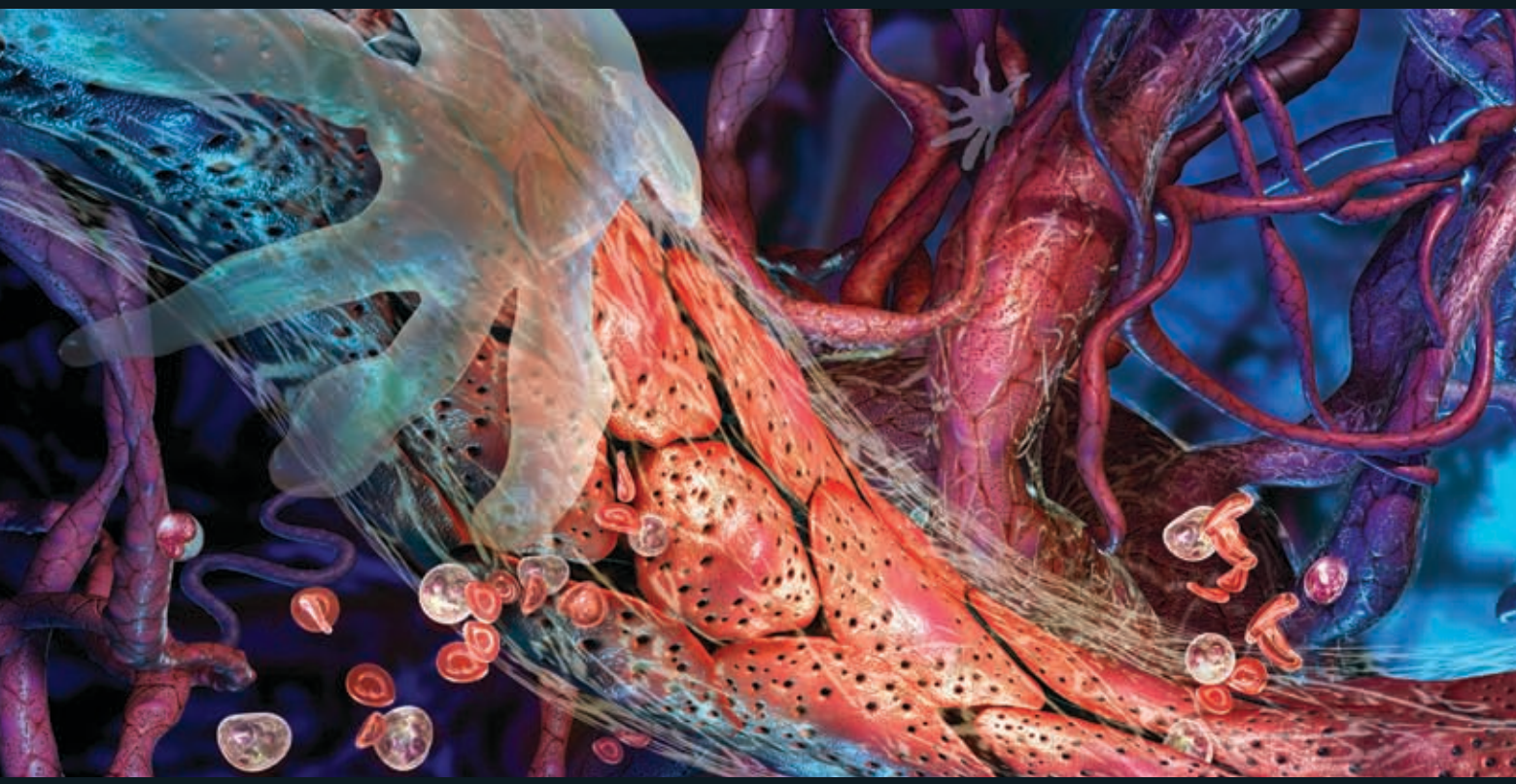

TAMING VESSELS TO TREAT CANCER

Restoring order to the chaotic blood vessels inside a tumor opens a window of opportunity for attacking it. Surprisingly, drugs meant to destroy vasculature can make the repairs and may help reverse conditions that lead to cardiovascular disease and blindness

By Rakesh K. Jain

While still a graduate student in 1974, I had a chance to see malignant tumors from a most unusual perspective. I was working at the National Cancer Institute in the laboratory of the late Pietro M. Gullino, who had developed an innovative experimental setup for studying cancer biology—a tumor mass that was connected to the circulatory system of a rat by just a single artery and a single vein. As a chemical engineer, I decided to use this opportunity to measure how much of a drug injected into the animal would flow to the tumor and back out again. Amazingly, most of the substance injected into the rat never entered the tumor. To make matters worse, the small amount that did reach the mass was distributed unevenly, with some areas accumulating hardly any drug at all.

My immediate concern was that even if a small fraction of the cancer cells in a human tumor did not

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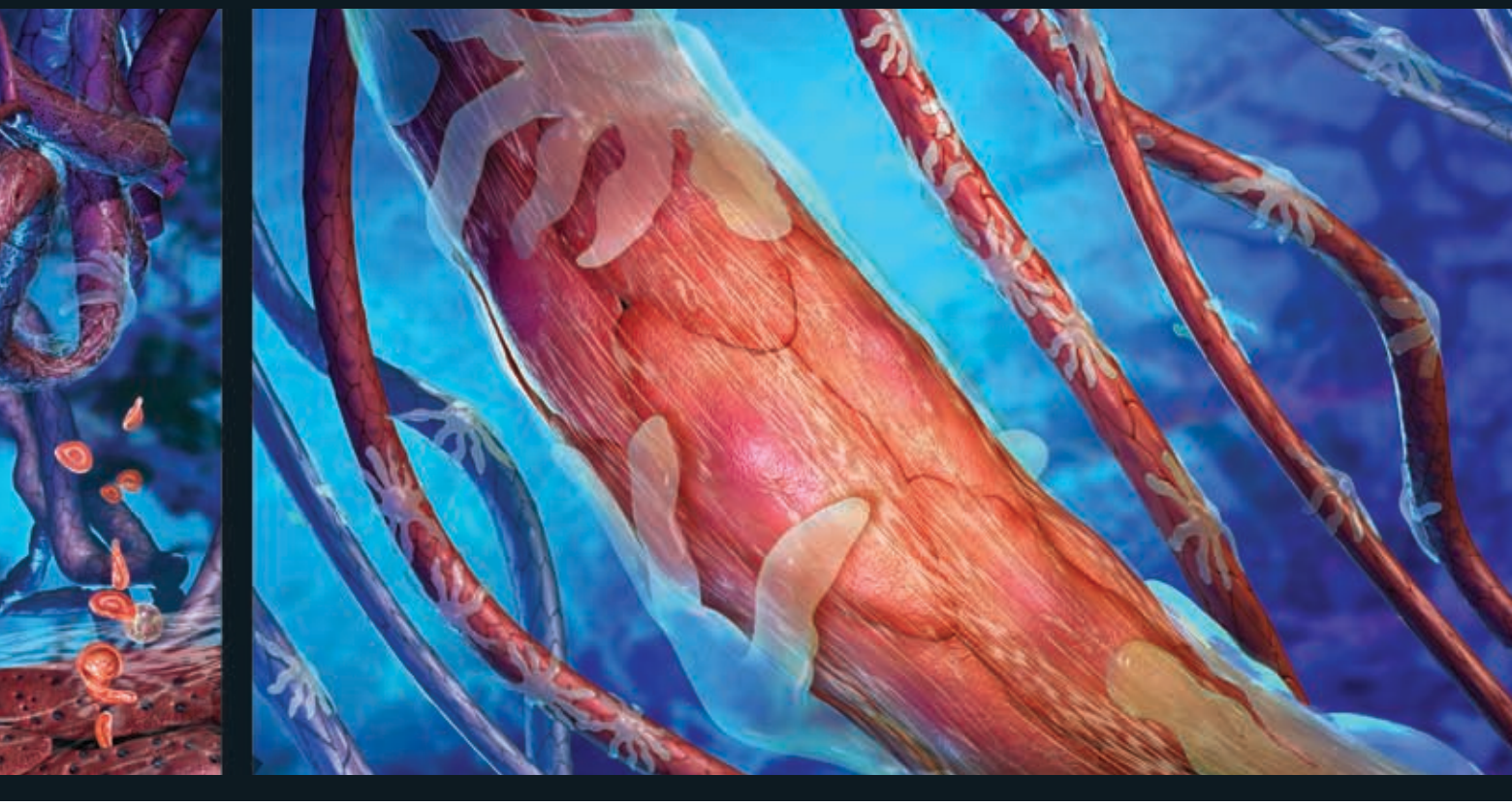

ABNORMAL blood vessels (*left*) add to the havoc inside a tumor and prevent treatments from reaching cancer cells. Normalizing those vessels (*above*) makes them into functional weapons that can be turned against the tumor.

KEITH KASNOT

receive an adequate dose of whatever anticancer drug was being applied, those cells could survive—causing the tumor to grow back sooner or later. Perhaps the engineer in me was also drawn to trying to understand and solve the apparent infrastructure problem inside tumors that posed a major obstacle to the delivery of cancer therapies.

Over the subsequent decades my colleagues and I have investigated what makes the vasculature within tumors abnormal and how these disordered blood vessels not only stymie traditional cancer treatments but also contribute directly to some of the malignant properties of solid cancers. Building on these insights, we developed approaches to normalizing tumor blood vessels and tested them successfully in mice. In the process, we also discovered a seeming paradox—a class of drugs designed to destroy the blood vessels of tumors actually acts to repair them, creating a window of opportunity to attack the cancer most effectively.

In recent years we have finally been able to start testing this idea in cancer patients, and the excitement in our lab was overwhelming when we saw the first clinical evidence of tumors shrinking in response to vascular normalization, just as we had anticipated. Much more work remains before we can perfect this therapeutic approach and gauge its usefulness in patients with different types of malignancy. But what we have already learned about restoring blood vessels is also opening doors to treating other vascular disorders, such as macular degeneration, the leading cause of blindness in the U.S.

Tortuous Road

THE JOURNEY THAT LED to our recent successes began in earnest a few years after I completed my doctoral studies. Determined to find out why drugs do not penetrate tumors uniformly, my colleagues and I started by monitoring every step of the process in rodents. Using a variety of techniques, we observed the progress of drugs as they entered the tiny blood vessels of a tumor, crossed the vessel walls into the surrounding tissue, entered into cancer cells and eventually exited the mass. Together with my students and collaborators, we developed methods for tracking mole-

Overview/*Controlling Chaos*

- Abnormal and dysfunctional blood vessels are a hallmark of solid tumors, one that contributes directly to malignant properties of a cancer as well as preventing treatments from reaching and attacking tumor cells.
- Normalizing tumor vessels allows cancer therapies to penetrate the mass and to function more effectively.
- Unexpectedly, drugs originally designed to destroy tumor blood vessels act to repair them for a time, opening a new avenue for cancer treatment as well as restoration of abnormal vasculature in other diseases.

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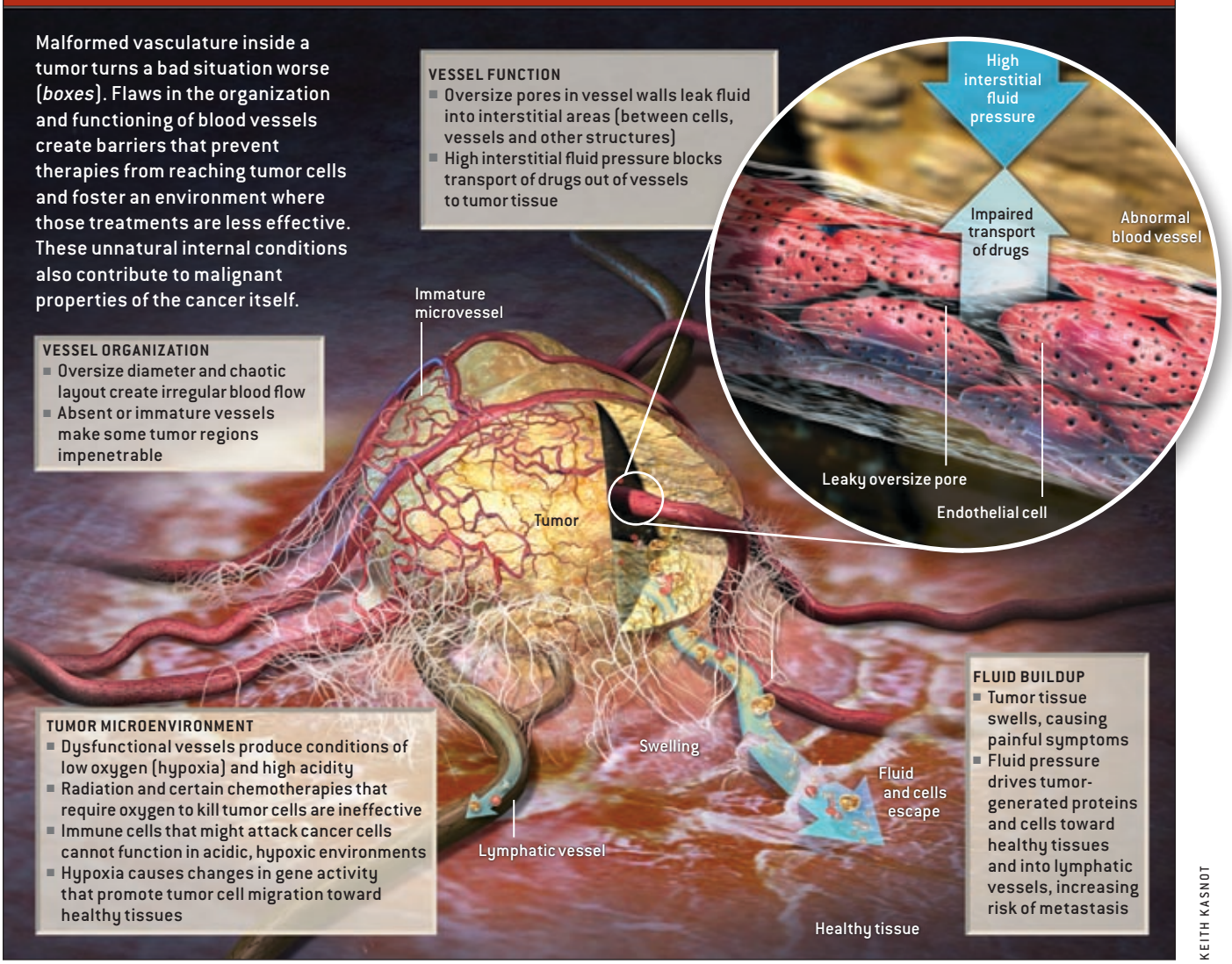
cules, such as oxygen, within blood vessels and tissues. Eventually we could even watch as genes turned on and off inside cells.

Early on it was apparent that the vessels within tumors bear little resemblance to normal ones. Healthy tissues are fed by straight vessels that branch predictably into successively smaller capillaries and microvessels, creating a pervasive network for delivering oxygen and nutrients to cells. Tumors, which stimulate the growth of new vasculature of their own, tend to generate a tangle of vessels. These connect to one another randomly, with some oversize branches, many extraneous immature microvessels and areas of a tumor that will lack vessels altogether.

Over the course of many years we managed to delineate the processes that govern the movement of fluids, drugs and cells within this tortuous vasculature and gained insight into the consequences of the abnormalities. The picture that emerged was grim: the very first thing we realized was that tumor blood vessels are not just disorganized in their appearance but highly aberrant in every aspect of their structure and function. We found that blood flows quite briskly in some vessels within a tumor, whereas it is static in others. In a given vessel, blood may travel in one direction for a while and then reverse direction. These flow patterns alone create a major obstacle to uniform drug delivery. Moreover, some parts of the vessel walls are overly leaky and others are unusually tight, which means that drugs and other molecules that managed to penetrate the vasculature would be distributed into the surrounding tumor tissue unevenly.

When we began investigating the causes of this nonuniform porousness, we discovered that in

ABNORMAL VESSELS MAKE TROUBLE


Malformed vasculature inside a tumor turns a bad situation worse (*boxes*). Flaws in the organization and functioning of blood vessels create barriers that prevent therapies from reaching tumor cells and foster an environment where those treatments are less effective. These unnatural internal conditions also contribute to malignant properties of the cancer itself.
VESSEL FUNCTION
Oversize pores in vessel walls leak fluid into interstitial areas (between cells, vessels and other structures)
High interstitial fluid pressure blocks transport of drugs out of vessels to tumor tissue
VESSEL ORGANIZATION
Oversize diameter and chaotic layout create irregular blood flow
Absent or immature vessels make some tumor regions impenetrable
TUMOR MICROENVIRONMENT
Dysfunctional vessels produce conditions of low oxygen (hypoxia) and high acidity
Radiation and certain chemotherapies that require oxygen to kill tumor cells are ineffective
Immune cells that might attack cancer cells cannot function in acidic, hypoxic environments
Hypoxia causes changes in gene activity that promote tumor cell migration toward healthy tissues
FLUID BUILDUP
Tumor tissue swells, causing painful symptoms
Fluid pressure drives tumor-generated proteins and cells toward healthy tissues and into lymphatic vessels, increasing risk of metastasis
High interstitial fluid pressure
Impaired transport of drugs
Abnormal blood vessel
Leaky oversize pore
Endothelial cell
Immature microvessel
Tumor
Swelling
Fluid and cells escape
Lymphatic vessel
Healthy tissue

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some tumors the pores in blood vessel walls could be as large as one or two microns in diameter, which is more than 100 times the size of pores in healthy vessels. As a result, these vessels are unable to maintain normal pressure gradients across their walls. Fluid pressure inside healthy blood vessels is typically much higher than in the surrounding tissue. Because tumor vessels are so porous, escaping fluid raises the outside—or interstitial—pressure until it nearly equals that inside the blood vessels.

BLOOD VESSELS in a normal mouse muscle's capillary bed (*left*) and inside a mouse tumor (*right*) differ distinctly. The tumor vessels branch erratically, vary in diameter along their lengths and are generally oversize—all features that contribute to irregular blood flow.

This unnatural pressure gradient is not just an impediment to the ability of drugs to reach tumor cells; the accumulation of interstitial fluid produces swelling in and around tumor tissues. In patients with brain cancers, where tissue expansion is limited by the skull, that swelling becomes a severe, often life-threatening problem in itself. In those with other types of cancer, the exuded fluid can also accumulate in body cavities. Wherever it goes, the fluid oozing from a tumor carries with it tumor cells as well as various tumor-generated proteins that promote the growth of new blood and lymphatic vessels in the surrounding normal tissue and lymph nodes—which can then serve as conduits for the metastatic spread of the cancer cells to other parts of the body.

Beyond the difficulty of delivering drugs through chaotic tumor vasculature and the dangerous fluid buildup caused by leaky vascular walls, the abnormalities of tumor vessels create a highly unnatural microenvironment inside a tumor as well. Because many areas of a tumor lack vasculature and existing vessels are unable to deliver sufficient oxygen to surrounding tissues, a general state of hypoxia (low oxygen) and high acidity prevails in the tumor. Hypoxia in turn makes tumor cells more aggressive and prone to metastasis. In addition, the body's immune cells, which might help fight a tumor, are hampered by acidity and cannot function in low oxygen. Nor can radiation treatments and a subset of chemotherapy drugs that depend on chemical processes that require oxygen to kill cancer cells.

Thus, what began as an inquiry into seemingly simple aberrations in the flow of drugs inside tumors revealed that the abnormalities in tumor blood vessels are obstacles to treatment in even more ways than I had initially imagined. By 1994 our observations were beginning to suggest to my research collaborators and me that if we knew how to repair the structure and function of tumor-associated blood vessels, we would have a chance to normalize the tumor microenvironment and ultimately improve cancer treatment. To accomplish such a reversal, we first had to gain a better understanding of what makes tumor vessels abnormal and keeps them that way.

Restoring Balance

WE BEGAN TO LOOK at the molecular factors involved in normal blood vessel formation, known as angiogenesis, including the single most potent one, vascular endothelial growth factor (VEGF). First discovered and named vascular permeability factor by my Harvard University colleague Harold Dvorak, VEGF promotes the survival and proliferation of endothelial cells, which form the inner lining of blood vessels. In excess, it also makes vessels leaky—hence its original name. In normal tissues, however, the collective action of VEGF and other growth-stimulating molecules like it is counterbalanced by the actions of natural antiangiogenesis molecules, such as thrombospondin, that inhibit blood vessel growth.

Whether healthy or diseased, tissues that need new blood vessels increase their production of angiogenesis stimulators or reduce their production of inhibitors, or do both, tipping the balance in favor of angiogenesis. In healthy processes such as wound healing, a balance between growth and inhibitory factors is eventually reinstated once the new vessels are established. But in tumors and a number of other chronic diseases, an imbalance persists—and blood vessels grow increasingly abnormal.

THE AUTHOR

RAKESH K. JAIN is Andrew Werk Cook Professor of Tumor Biology and director of the Edwin L. Steele Laboratory for Tumor Biology in the radiation oncology department of Massachusetts General Hospital and Harvard Medical School. His research incorporates biology, imaging, engineering and mathematics in the study of blood and lymphatic vessels and their tissue environment, as well as the adaptation of basic findings to patient treatment. He would especially like to acknowledge the National Cancer Institute for continuous support of his work since 1980 and more than 200 graduate students, postdoctoral fellows and collaborators worldwide who have shared his journey into the world of solid tumors. Jain also serves as an adviser to several pharmaceutical and biotechnology companies and is a member of both the National Academy of Engineering and the Institute of Medicine.

FROM "MOLECULAR REGULATION OF VESSEL MATURATION," BY RAKESH K. JAIN, IN *NATURE MEDICINE*, VOL. 9, 2003, REPRINTED WITH PERMISSION OF MACMILLAN PUBLISHERS LTD.

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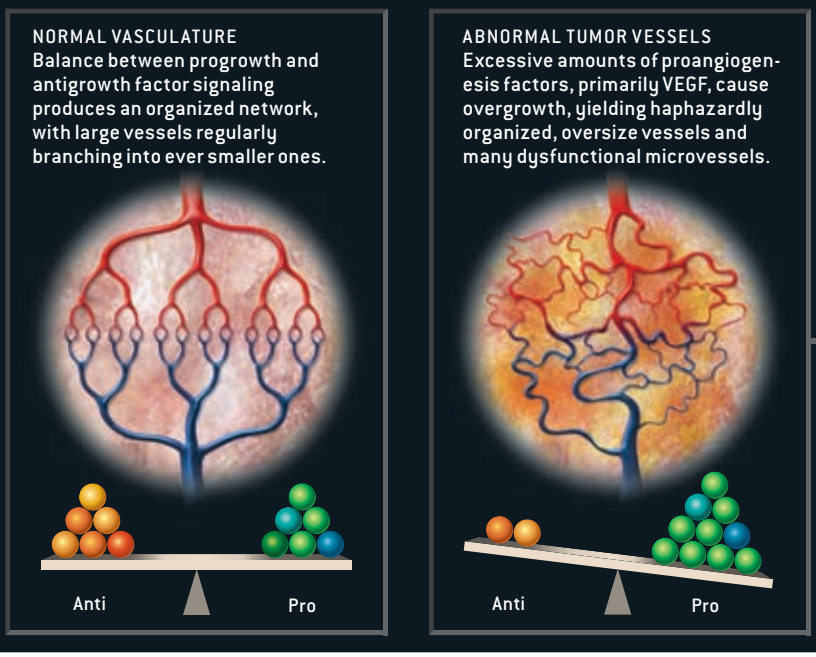
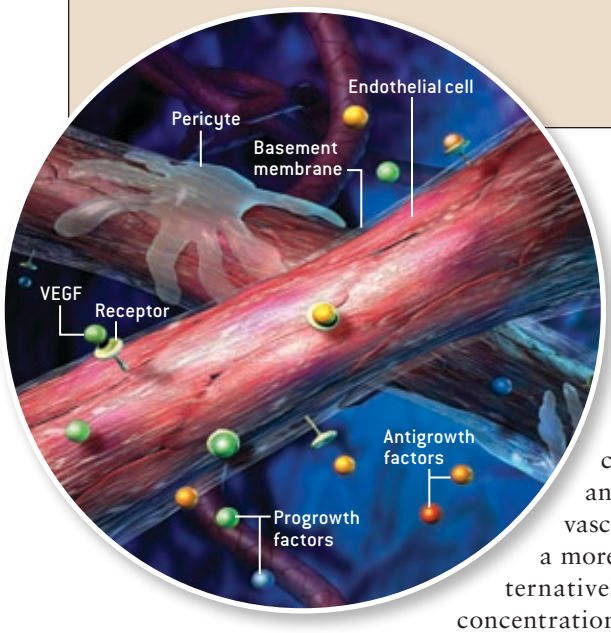
TIPPING THE BALANCE TOWARD NORMALCY

Restoring tumor vasculature to a more normal state optimizes conditions for anticancer therapies to reach tumor cells and to work more effectively. Many abnormalities arise in tumor vessels because factors that stimulate vessel growth, or angiogenesis, overwhelm growth inhibitors that usually check vessel proliferation. Antiangiogenesis drugs that suppress the primary progrowth factor, VEGF, tip the balance back toward normal vessel formation and maintenance. Normalization does not last indefinitely, because the drugs may ultimately destroy most vessels or a tumor may become resistant to them.


NORMAL VASCULATURE
Balance between progrowth and antigrowth factor signaling produces an organized network, with large vessels regularly branching into ever smaller ones.
Anti
Pro
ABNORMAL TUMOR VESSELS
Excessive amounts of proangiogenesis factors, primarily VEGF, cause overgrowth, yielding haphazardly organized, oversize vessels and many dysfunctional microvessels.
Anti
Pro

▼ **HEALTHY VESSEL GROWTH AND MAINTENANCE**
Endothelial cells form blood vessels in response to signals from molecules that promote and inhibit growth. Pericyte cells straddle vessels, and a basement membrane surrounds them; both provide support.



Because VEGF is abundant in most solid tumors, I suspected that finding a way to mop up the excess VEGF or interfere with the growth signals it generates could restore balance and cause tumor vasculature to revert to a more normal state. Alternatively, increasing the concentration of angiogenesis-inhibiting factors could have the same normalizing effect on the blood vessels. I also theorized that vessels treated in either way would not remain normal forever—they would be destroyed if the inhibitors were potent enough or would become abnormal again if the tumors developed the ability to make different stimulators, such as basic fibroblast growth factor (bFGF), which can mimic many of the effects of VEGF. The only way to find out was to try angiogenesis inhibitors on tumors and see what happened.

In 1995 antibody-based drugs that could neutralize the effects of VEGF were already in development, so we were able to use these to test our approach in mice. Certain of the antibodies attached directly to VEGF, hindering its ability to send a growth signal to endothelial cells by binding to receptors on the cell surface. Other antibodies bound to the VEGF receptors themselves, preventing the growth factor from making contact. Remarkably, both forms of VEGF inhibition caused some of the immature and inefficient blood vessels characteristic of many tumors to be pruned away and induced the remaining vessels to remodel themselves so that they began to resemble normal vasculature. Those normalized blood vessels were less leaky, less dilated and less tortuous. We could also detect functional improvements in the tumors, including lower interstitial fluid pressure, higher oxygenation and improved penetration of drugs.

As excited as we were by these results and by the fact that they were later reproduced in animals by other researchers, we still could not know whether the same responses would occur in cancer patients. And many researchers were understandably skeptical of our approach. By the late 1990s, when I first proposed the idea of tumor vessel normalization publicly, scientists in academia and industry had been working on making drugs to destroy blood vessels. Their pursuit was based on the hypothesis put forward in 1971 by the late Judah Folkman, my former Harvard colleague, that tumor growth could be halted by starving the mass using antiangiogenic drugs. Indeed, the drug Avastin, approved by the Food and Drug Administration for use in cancer treatment in 2004, is a VEGF-neutralizing antibody originally developed as such an antiangiogenesis agent.

In laboratory testing and clinical trials, Avastin

KEITH KASNOT (*illustrations*); JEN CHRISTIANSEN (*seesaws*); ADAPTED FROM ORIGINAL ILLUSTRATIONS BY LANCE L. MUNN IN "NORMALIZING TUMOR VASCULATURE WITH ANTI-ANGIOGENIC THERAPY: A NEW PARADIGM FOR COMBINATION THERAPY," BY RAKESH K. JAIN, IN *NATURE MEDICINE*, VOL. 7, NO. 9, SEPTEMBER 2001

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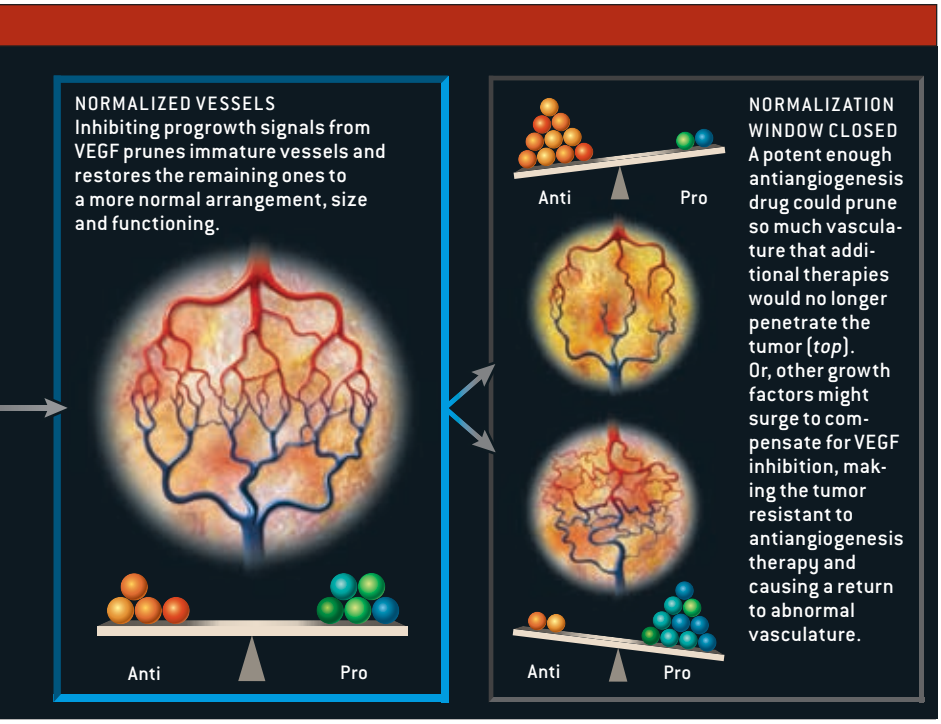

NORMALIZED VESSELS
Inhibiting progrowth signals from VEGF prunes immature vessels and restores the remaining ones to a more normal arrangement, size and functioning.
Anti
Pro
Anti
Pro
NORMALIZATION WINDOW CLOSED
A potent enough antiangiogenesis drug could prune so much vasculature that additional therapies would no longer penetrate the tumor (*top*).
Or, other growth factors might surge to compensate for VEGF inhibition, making the tumor resistant to antiangiogenesis therapy and causing a return to abnormal vasculature.
Anti
Pro

has been shown to destroy blood vessels in animal and human tumors, although when used alone it does not increase overall survival in cancer patients. In a pivotal clinical trial that led to its approval, however, Avastin did increase the survival of patients with advanced colorectal cancer but *only* when it was used in conjunction with standard chemotherapy. That positive outcome seemed quite paradoxical at the time because, in principle, a drug that was designed and deployed to destroy blood vessels should reduce the effectiveness of chemotherapy, which requires functioning blood vessels to reach tumor cells. Some published studies have in fact shown that antiangiogenic agents can hinder radiation and chemotherapy. So how could these apparently contradictory findings be reconciled?

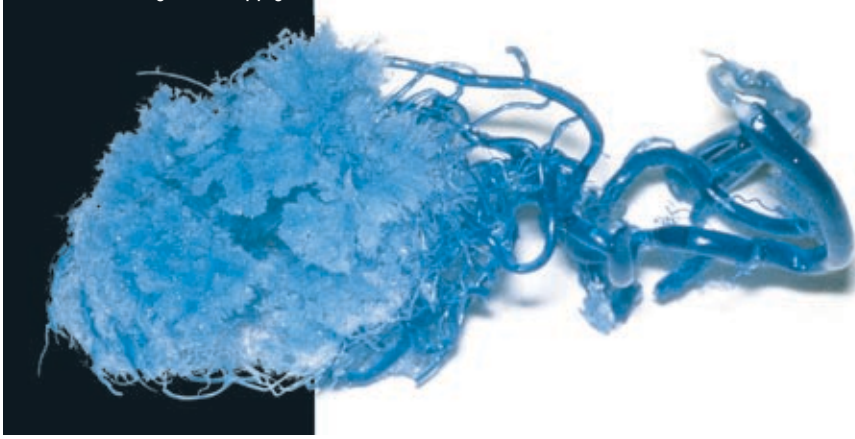
Our group had the chance to find out by closely examining the structure and function of blood vessels in the tumors of rectal cancer patients receiving Avastin and combined chemotherapy and radiation in a 2002 clinical trial supported by the National Cancer Institute and led by Christopher Willett, now at Duke University Medical Center. Very quickly, we saw that the changes to tumor vasculature in those patients were not limited to simple vessel destruction.

Two weeks after a single injection of Avastin, blood flow within the tumors did drop by 30 to 50 percent in six consecutive patients. The density of microvessels, the overall number of blood vessels and the interstitial fluid pressure in the tumors were all reduced as well. And a form of programmed cell death known as apoptosis, characteristic of oxygen and nutrient deprivation, increased among tumor cells that no longer had access to the pruned vasculature.

Surprisingly, however, there was no concurrent decrease in a sign of overall energy use by the tumor, its uptake of a glucose analogue, as might be expected if the tumor were only being starved. Instead it seemed that the remaining tumor vessels had become more efficient in supporting the energy needs of the surviving cancer cells. Furthermore, the rate of proliferation of cancer cells increased in some tumors, reflecting their access to better-functioning vessels and a more normal tissue microenvironment. Although increased proliferation is usually not desirable when it comes to cancer cells, that state would make them more sensitive to chemotherapy drugs, which generally target dividing cells.

Together these results provided a first glimpse of how a drug such as Avastin works in patients and thereby revealed why it might improve the outcome of radiation or chemotherapy for a time. As the drug blocks the effects of VEGF, some tumor vasculature is pruned away immediately, but the vessels that remain become less abnormal. In addition to improving the overall tumor microenvironment, those normalized vessels also make the surviving cells more vulnerable to the treatments that they can now deliver more efficiently. Restor-

TUMOR VESSELS cast in polymer from a surgically excised colon cancer form a dense crystalline mass representing a chaotic profusion of microvessels. In contrast, large gaps indicate areas of the tumor that lacked any blood supply.



POLYMER CAST FROM "GEOMETRIC RESISTANCE AND MICROVASCULAR NETWORK ARCHITECTURE OF HUMAN COLORECTAL CARCINOMA," BY J. R. LESS, M. C. POSNER, T. C. SKALAK, N. WOLMARK AND R. K. JAIN, IN *MICROCIRCULATION*, VOL. 4, NO. 1; 1997

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ing normal function to tumor vessels thereby creates a period during which treatment with a variety of cancer therapies should be maximally effective.

Window of Opportunity

TO TRULY BENEFIT from this new insight into the way that antiangiogenic therapy can work with radiation or chemotherapies, an oncologist would need to know when a patient's tumor vessels first begin to normalize and how long they remain that way. My research group returned to experimenting with mice to better characterize this period we came to call the "normalization window." We treated brain tumors in the animals with an antibody designed to block the main VEGF receptor used by endothelial cells and saw signs of vessel normalization begin after one day. During the normalization window—which lasted only about five to six days—tumor oxygenation increased and radiation therapy yielded the best therapeutic outcome. Other teams working with laboratory animals have subsequently reported similar observations.

Enough evidence supported this model, in fact, that we were able to test it in another National Cancer Institute clinical trial, completed in late 2006. Led by my Massachusetts General Hospital colleagues Tracy Batchelor and Gregory Sorensen, the trial included 30 patients whose brain tumors, known as glioblastomas, had regrown despite aggressive surgery, radiation and chemotherapy. These patients had a life expectancy of less than six months.

They received a daily oral dose of Recentin, an experimental drug that potently inhibits the three primary cellular receptors for VEGF. Using advanced imaging techniques, we were able to look for effects in their tumors and saw them almost immediately [*see illustration on opposite page*]. The signs of vascular normalization included reduced vessel diameter and leakiness, which continued for at least 28 days, with some normalized characteristics persisting for the entire four-month duration of the study. Moreover, as anticipated by our original model, the normalization was accompanied by a rapid decrease in swelling in and around the tumor, an effect that continued as long as the patients took the Recentin. Because the side effects of VEGF inhibition can be severe, however, some patients asked for a break from the treatment during the trial, which allowed us to observe the tumor vessels becoming abnormal again when Recentin was discontinued and renormalized when the drug was resumed.

These results were the first to define how long the period of vascular normalization can last in humans and have led to a much larger ongoing clinical trial involving 300 patients to further define the role that Recentin, with and without chemotherapy, might play in the treatment of glioblastoma. We are also studying a number of antiangiogenic drugs in combination with traditional therapies in newly diagnosed and recurrent tumor cases in more types of cancer.

At the same time, we are also investigating ways of expanding the window of normalization so that survival improvements can be extended from months to years. Any potential strategy to repair vessels must recognize that VEGF blockade alone may not always be sufficient to achieve or sustain normalization, because tumors can substitute other growth factors to get around the loss of VEGF signaling. As

KANG ZHANG AND JAMES GILMAN

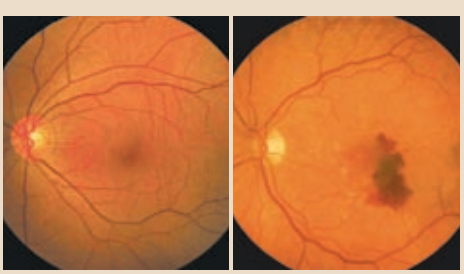
Vessel Repair: Beyond Cancer

Hundreds of millions of people around the world suffer from noncancerous conditions that involve abnormal vasculature. Modifying blood vessel growth and function might become a key component of the therapeutic arsenal for those diseases as well, so drugs that normalize blood vessels have the potential to vastly impact human health.

Among the most widespread of problems in this category, for example, is atherosclerosis, an artery disease whose features include an accumulation of fatty plaques within the inner walls of blood vessels. Inside such plaques, inflammation-inducing blood cells and other detritus accumulate, gradually enlarging the lesion. New blood vessels sprout within this growing mass to feed it, much like a tumor. These new vessels also share many abnormal features with tumor vessels, such as leakiness and disorganization. In principle, therefore, applying antiangiogenic agents should normalize intraplaque vessels, stabilizing the lesions, halting their expansion and reducing their potential for rupture.

Eye diseases such as diabetic retinopathy and the so-called wet form of age-related macular degeneration (AMD) are also characterized by vascular abnormalities similar to those seen in tumors. A hallmark of wet AMD is, in fact, the leakiness of blood vessels in the retina at the back of the eye. As a result, blood oozes into surrounding tissue, causing partial or total vision loss. More than nine million Americans are currently affected. Not surprisingly, the greatest progress outside the realm of cancer treatment in using antiangiogenesis to repair vascular abnormalities has been in wet AMD. Two drugs, Lucentis and Macugen—both inhibitors of VEGF—have already been approved for treating the condition and most likely work by normalizing the leaky vessels.

These same normalization principles may also be useful for controlling conditions that cause fluid buildup (edema) and for tissue engineering and regenerative medicine, which require the creation and maintenance of normally functioning vasculature. —*R.K.J.*



WET AMD affects the macula, a region of the retina at the back of the eye that appears slightly darkened in a healthy human eye (*left*) but can sprout excess blood vessels leading to macular degeneration. The abnormal vessels leak blood (*right*) into surrounding tissue, obscuring vision.

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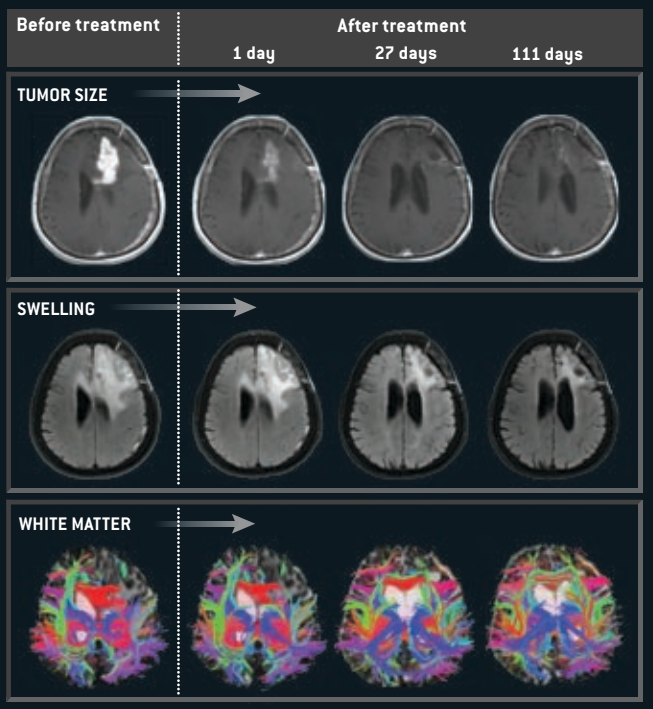

BRAIN TUMOR response to antiangiogenesis therapy reflects some effects of vascular normalization. MRI images of a patient's brain one day before beginning treatment and at subsequent intervals show a shrinking white area corresponding to the malignant tumor's location (*top row*). Another set of views highlights accumulated fluid in the same area receding (*middle row*). As tumor-generated edema diminishes, reduced compression of brain tissue is visible in the rebounding of fibrous white matter tracts, which are color-coded here by brain region (*bottom row*).

tumors grow larger, for instance, they tend to make a diverse array of proangiogenic molecules in addition to VEGF, so their vessels may gradually lose responsiveness to a treatment such as Avastin.

In rectal cancer patients, for instance, our group discovered that blood levels of VEGF and PlGF (placental growth factor), a related molecule, actually increased after VEGF was mopped up with Avastin, suggesting that the tumor or other tissues began manufacturing more of those factors in response. And in recurrent glioblastoma patients, blood levels of multiple proangiogenic molecules rose as tumors escaped the Recentin treatment.

This diversification of progrowth signals illustrates that the challenge for the oncologist will be to formulate cocktails of agents specifically tailored to the molecular profile of each patient's primary and metastatic tumors and to changes in those profiles that will likely occur over time. It is worth noting, however, that the available tools for promoting vascular normalization are not limited to drugs targeting VEGF or other growth factors directly. We have shown in mice, for example, that the drug Herceptin—an antibody that targets a tumor cell-surface protein called HER2 and that is given to about a quarter of women with breast cancer—can mimic the responses produced by an antiangiogenic cocktail and normalize tumor vessels. Herceptin indirectly lowers cellular manufacture of several proangiogenic molecules while increasing the cells' production of the antiangiogenic thrombospondin-1.

In addition to identifying new and existing medications that can foster vascular normalization, it will be important to find minimally invasive and affordable ways for doctors to monitor the normalization process, to best exploit it when delivering treatments. To that end, my colleagues and I have been working to identify so-called biomarkers: readily identifiable signs that reflect what is happening inside the tumor and thereby reveal the onset and duration of the normalization window in individual patients. Such markers might include, for example, proteins in the bloodstream or in urine whose levels rise or fall during this time window.

Finding that antiangiogenesis drugs can normalize vasculature should not suggest that the original purpose for which they were developed is no longer valid. If a drug is sufficiently potent and specific to destroy enough tumor vasculature to starve the entire tumor and save a patient's life, then that would be a happy outcome for everyone. But the ability to use these drugs for vascular repair as well makes them valuable tools for attacking tumors in more than one way. In the longer term, this research can also benefit the many millions of people around the world suffering from other diseases caused by abnormal vasculature, such as age-related macular degeneration and atherosclerosis [*see box on opposite page*].

More than 30 years ago, when I first set out to understand the tortuous and dysfunctional blood vessels of tumors, I never imagined where that road would lead. Nor could I have pictured a day when a patient with a disease of abnormal blood vessels could walk into a clinic, have various biomarkers measured, then receive a tailored regimen of normalizing drugs to repair those vessels. But now that day looks closer than ever before. SA

MORE TO EXPLORE

Normalization of the Tumor Vasculature: An Emerging Concept in Anti-Angiogenic Therapy. Rakesh K. Jain in *Science,* Vol. 307, pages 58–62; January 2005.

Lessons from Phase III Clinical Trials of Anti-VEGF Therapy for Cancer. Rakesh K. Jain, Dan G. Duda, Jeffrey W. Clark and Jay S. Loeffler in *Nature Clinical Practice Oncology,* Vol. 3, No. 1, pages 24–40; January 2006.

Angiogenesis in Brain Tumors. Rakesh K. Jain et al. in *Nature Reviews Neuroscience,* Vol. 8, pages 610–622; August 2007.

Antiangiogenic Therapy for Normalization of Atherosclerotic Plaque Vasculature: A Potential Strategy for Plaque Stabilization. Rakesh K. Jain et al. in *Nature Clinical Practice Cardiovascular Medicine,* Vol. 4, No. 9, pages 491–503; September 2007.

For more articles by Rakesh K. Jain and animations explaining vessel normalization, go to **http://steele.mgh.harvard.edu**

FROM "AZD2171, A PAN-VEGF RECEPTOR TYROSINE KINASE INHIBITOR, NORMALIZES TUMOR VASCULATURE AND ALLEVIATES EDEMA IN GLIOBLASTOMA PATIENTS," BY T. BATCHELOR ET AL., IN *CANCER CELL*, VOL. 11, JANUARY 2007, © 2007 REPRINTED WITH PERMISSION OF ELSEVIER

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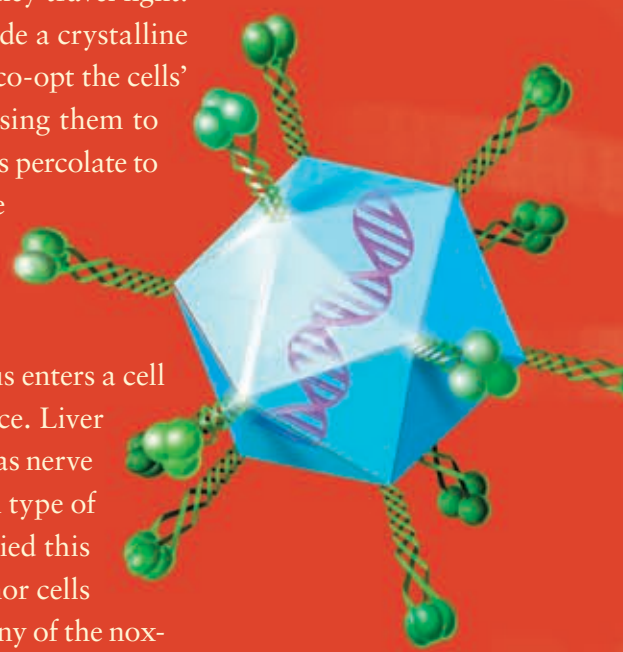
TUMOR-BUSTING

A technique called virotherapy harnesses viruses, those banes of humankind, to stop another scourge—cancer

By Dirk M. Nettelbeck, Ronald D. Alvarez and David T. Curiel



Viruses are some of the most insidious creations in nature. They travel light: equipped with just their genetic material packed tightly inside a crystalline case of protein, they latch onto cells, insert their genes, and co-opt the cells' energy-producing, gene-copying and protein-making machinery, using them to make thousands of copies of themselves. Once formed, the new viruses percolate to the cell surface, pinch off inside minuscule bubbles of cell membrane and drift away, or else they continue reproducing until the cell finally bursts. In any case, they go on to infect and destroy other cells, resulting in diseases from AIDS to the common cold.

Different viruses cause different diseases in part because each virus enters a cell by first attaching to a specific suction cup–like receptor on its surface. Liver cells display one kind of receptor used by one family of viruses, whereas nerve cells display another receptor used by a different viral family, so each type of virus infects a particular variety of cell. Cancer researchers have envied this selectivity for years: if they could only target cancer therapies to tumor cells and avoid damaging normal ones, they might be able to eliminate many of the noxious side effects of cancer treatment.

Some scientists, including ourselves, are now genetically engineering a range of viruses that act as search-and-destroy missiles: selectively infecting and killing cancer cells while leaving healthy ones alone. This new strategy, called virotherapy, has shown promise in animal tests, and clinical trials involving human patients are now under way. Researchers are evaluating virotherapy alone, as well as viruses "armed" with therapeutic genes that enable them to administer traditional chemotherapies solely to tumor cells. They are also developing methods to label viruses, or the cells infected by these viruses, to track the movement of the viral agents in patients.

One of the first inklings that viruses could be useful in combating cancer came in 1912, when an Italian gynecologist observed the regression of cervical cancer in

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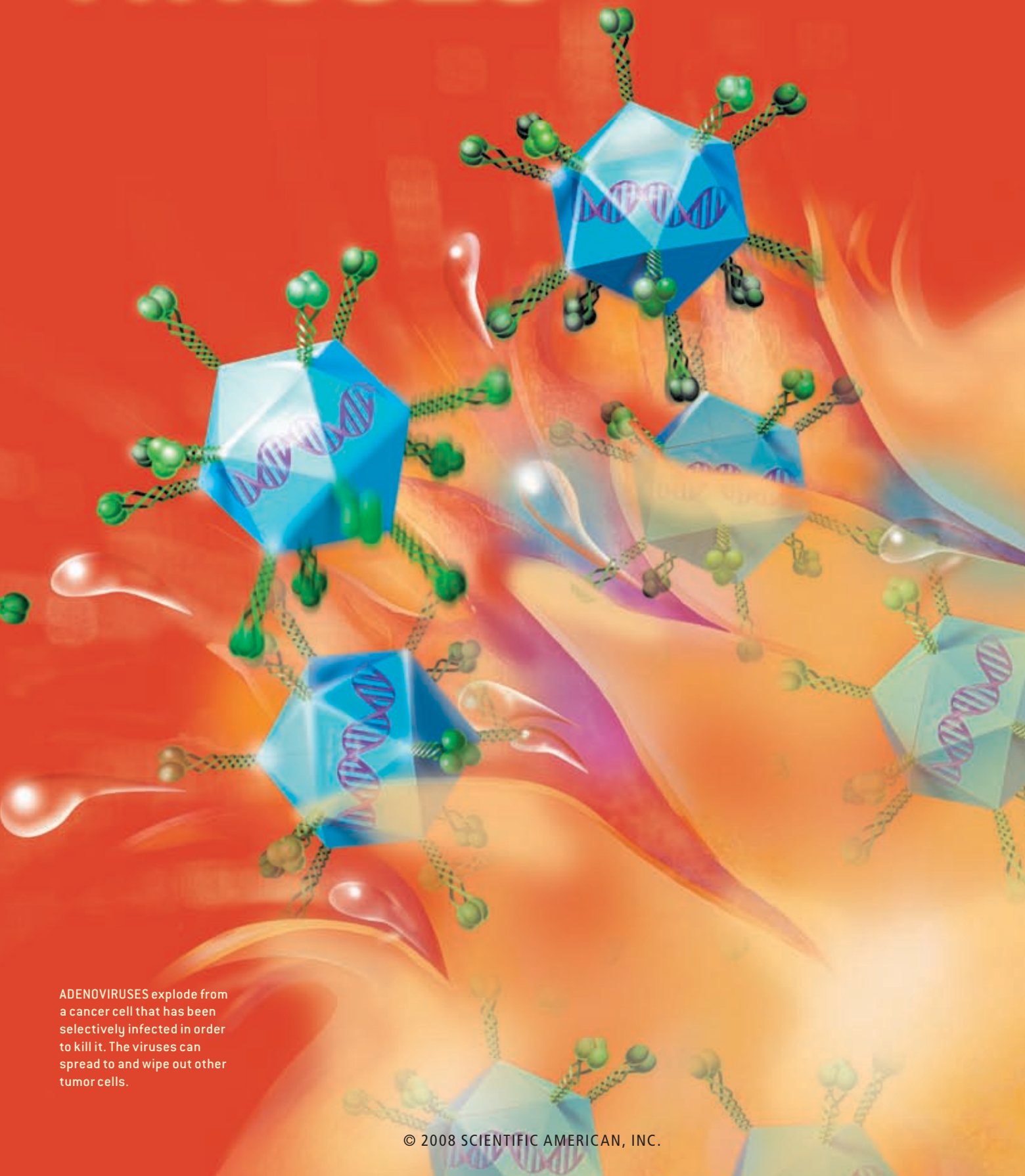
VIRUSES



ADENOVIRUSES explode from a cancer cell that has been selectively infected in order to kill it. The viruses can spread to and wipe out other tumor cells.

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a woman who was inoculated with a rabies vaccine made from a live, crippled form of the rabies virus. Physicians first injected viruses into cancer patients intentionally in the late 1940s, but only a handful appeared to benefit. Twenty years later scientists found that a virus that causes the veterinary disorder Newcastle disease shows a preference for infecting tumor cells and began to try to enhance that tendency by growing the viruses for generations in human cancer cells in laboratory culture dishes. Although critics countered that such viruses could be exerting only an indirect effect against cancer by generally activating an individual's immune system and making it more likely to detect and kill cancer cells, reports continued to pop up in the medical literature linking viral infection and cancer remission. In the early 1970s and 1980s two groups of physicians described patients whose lymphomas shrank after they came down with measles.

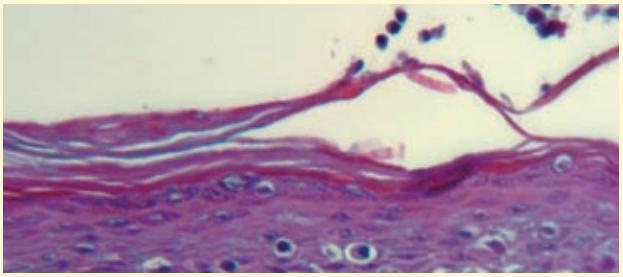
The modern concept of virotherapy began in the 1990s, when researchers genetically modified viruses to selectively replicate in, and kill, tumor cells. Teams led by Frank McCormick of ONYX Pharmaceuticals in Richmond, Calif., and Daniel R. Henderson of Calydon in Sunnyvale, Calif., independently published reports showing they could target virotherapy to human cancer cells grafted into mice, thereby eliminating the human tumors. (ONYX is no longer developing therapeutic viruses, and Calydon has been acquired by Cell Genesys in South San Francisco, Calif.) Both groups used derivatives of an adenovirus, a cause of the common cold that has been intensively explored for virotherapy. (Other viruses under study include herpes simplex, measles, parvovirus, vaccinia, reovirus and the avian Newcastle disease virus.) Adenovirus is appealing in part because researchers understand its biology very well after years of trying to cure colds and of using the virus in molecular biology and gene therapy research. It consists of a 20-sided protein case, or capsid, filled with DNA and equipped with 12 protein "arms." These protrusions have evolved over millennia to latch onto a cellular receptor whose normal function is to help cells adhere to one another.

Overview/*Anticancer Viruses*

- Virotherapy is a strategy to treat cancer by selectively infecting and killing tumor cells. Researchers are testing various approaches to target viruses to cancer cells, leaving normal cells untouched.
- The viruses used in virotherapy can kill tumor cells either by bursting them open or by combining virus replication with the delivery of genes that make the cells more susceptible to traditional chemotherapies.
- The same types of viruses used in virotherapy can also be labeled with fluorescent tags or devices for detection by nuclear medicine. Once delivered into the body, they home in on cancer cells. In the future, physicians might be able to use this imaging technique to detect the presence of tiny tumors.

TARGETING MELANOMA

The skin cancer melanoma can be highly lethal unless detected early; it arises from pigment cells in the skin or eye, called melanocytes, by uncontrolled growth and spread. Scientists are using the virotherapy approach to selectively kill melanoma cells while leaving healthy cells alone. One technique for studying melanoma involves combining melanoma cells



Adenoviruses are distinct from the types of viruses usually used in gene therapy to treat inherited disorders. Gene therapy traditionally employs retroviruses to splice a functioning copy of a gene permanently into the body of a patient in whom that gene has ceased to work properly. Unlike retroviruses, however, adenoviruses do not integrate their DNA into the genes of cells they infect; the genes they ferry into a cell usually work only for a while and then are lost. Scientists have investigated adenoviruses extensively in gene therapy approaches to treat cancer, in which replication-defective derivatives of the viruses are exploited as vectors for transferring into cells genes that, for example, make cancer cells more susceptible than normal ones to chemotherapy. In general, tests involving adenovirus vectors have been safe, but regrettably a volunteer died in 1999 after receiving an infusion of adenoviruses as part of a clinical trial to test a potential gene therapy for a genetic liver disorder [*see box on page 78*].

Gene therapists have been working to tailor adenoviruses and other viral vectors, or gene-delivery systems, to improve their safety and reduce the chances that such a tragedy might occur again. It is perhaps even more essential for researchers such as ourselves, who are investigating virotherapy, to develop safer, more targeted vectors, because virotherapy by definition aims to kill the cells the viruses infect and thereby produce a new generation of infectious viruses, not just insert a therapeutic gene into them. Replicating in the wrong cells could be dangerous.

Homing In on the Target

VIROTHERAPISTS ARE DEVISING two main strategies to make sure their missiles hit their objectives accurately with no collateral damage. In the first approach, termed transductional targeting, researchers are attempting to adapt the viruses so that they preferentially enter, or transduce, cancer cells. The second method, called transcriptional targeting, involves altering the viruses so that their genes can be active, or transcribed, only in tumors [*see box on pages 76 and 77*].

Transductional targeting is particularly necessary because

ILLUSTRATION BY TERESE WINSLOW (*preceding pages*)

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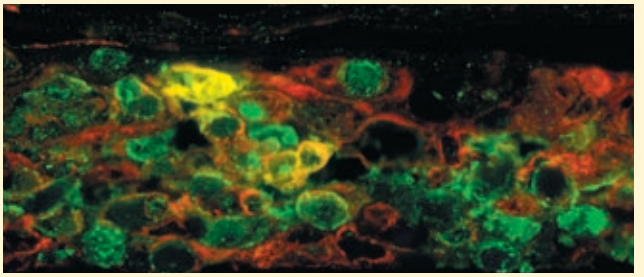
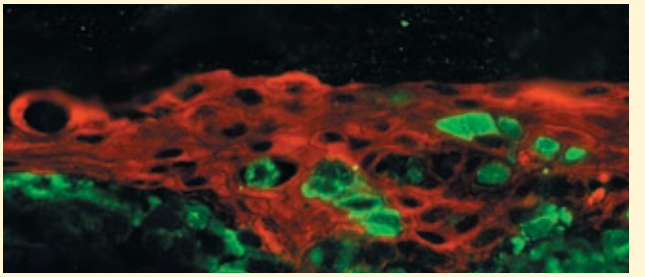
(*dark dots in micrograph below left*) with normal skin cells called keratinocytes and collagen to make cancer-bearing artificial skin that can be grown in laboratory culture dishes. One of us (Nettelbeck) and colleagues have devised an adenovirus that can specifically reproduce in melanoma cells. In the center and right micrographs of fluorescently labeled artificial skin (*below*), healthy keratinocytes appear red; viral proteins in cells show up green. The center micrograph was made using viruses that were not specifically targeted to melanomas. The viruses were able to grow in healthy cells, making those cells look yellow. In contrast, the targeted virus (*below right*) did not replicate in healthy cells, so none of the cells are yellow. —*D.M.N., R.D.A. and D.T.C.*





DIRK M. NETTELBECK AND N. SANJIB BANERJEE, THOMAS R. BROKER AND LOUISE T. CHOW *University of Alabama at Birmingham (micrographs)*

viruses have not evolved to infect and kill tumor cells. Unfortunately, adenoviruses bind more efficiently to the variety of normal tissues in the human body than they do to most tumor cells. We can reverse this pattern using specially generated adapter molecules made of antibodies that snap onto the arms of the virus like sockets on a socket wrench. By attaching carefully chosen antibodies or other molecules that selectively bind only to a specific protein found on tumor cells, we can render adenoviruses unable to infect any cells but cancerous ones. Once the antibody-bearing virus latches onto a targeted cell, the hapless cell engulfs it in a membrane sac and pulls it inside. As the sac disintegrates, the viral capsid travels to a pore in the cell's nucleus and injects its own DNA. Soon the viral DNA directs the cell to make copies of the viral DNA, synthesize viral proteins and combine the two into thousands of new adenoviruses. When the cell is full to capacity, the virus prompts the cell to burst, releasing the new viruses to spread to other cells.

The viruses can also be engineered more directly. In this regard, Curiel's group at the University of Alabama's Gene Therapy Center has designed adenoviruses that bind to cellular proteins called integrins. These molecules help cells stick to the network of connective tissue, called the extracellular matrix, that organizes the cells into cohesive tissues. Although integrins are also made by healthy cells, cancer cells produce them in abundance as they become metastatic and begin to squeeze through tissue layers and travel throughout the body. The University of Alabama research group has had encouraging results using the engineered viruses in mice bearing human ovarian cancers. The viruses homed in on the ovarian tumor cells and killed them, ridding the treated animals of the disease.

Transcriptional targeting generally takes advantage of genetic switches (promoters) that dictate how often a given gene is functional (gives rise to the protein it encodes) in a particular type of cell. Although each body cell contains the same encyclopedia of genetic information, some cells use different chapters of the encyclopedia more often than others to fulfill their specialized tasks. Skin cells called melanocytes, for instance, must make much more of the pigment melanin than do liver cells, which have little use for the protein. Accordingly, the promoter for the key enzyme for making melanin gets turned on in melanocytes but generally is off in most other body tissues. In the deadly skin cancer melanoma, the gene encoding this enzyme is frequently fully functional, making the tumors appear black. We, and others, have engineered adenoviruses that have a promoter for the enzyme adjacent to genes that are essential for the viruses' ability to replicate. Although these viruses might enter normal cells, such as liver cells, they can reproduce only inside melanocytes, which contain the special combination of proteins needed to turn on the promoter.

Researchers are currently tailoring adenoviruses with a variety of promoters that limit their activity to particular organs or tissues. In liver cancers, for example, the promoter for the gene α-*fetoprotein*—which is normally shut down after fetal development—becomes reactivated. Adenoviruses containing that same promoter hold promise for eradicating liver tumors. Scientists led by Jonathan W. Simons, now at the Prostate Can-

THE AUTHORS

DIRK M. NETTELBECK heads a research group focusing on virotherapy at the German Cancer Research Center and at Heidelberg University Hospital. He was a molecular biologist and postdoctoral fellow of the German Research Association at the University of Alabama at Birmingham (U.A.B.) from 2000 to 2003. He received his Ph.D. in 2000 from Philipps University in Marburg, Germany. *RONALD D. ALVAREZ* is professor in and director of the division of gynecologic oncology at U.A.B., and he directs the Women's Cancer Program of the U.A.B. Comprehensive Cancer Center. He received his M.D. from Louisiana State University in 1983. He has extensive experience in translational gene therapy research for ovarian cancer. *DAVID T. CURIEL* is director of the division of human gene therapy at the Gene Therapy Center at U.A.B. Curiel, who holds an M.D. and a Ph.D., is Jeanne and Anne Griffin Chair for Women's Cancer Research at U.A.B. and a professor of gene therapy at the Free University of Amsterdam. He has an equity interest in Birmingham-based VectorLogics.

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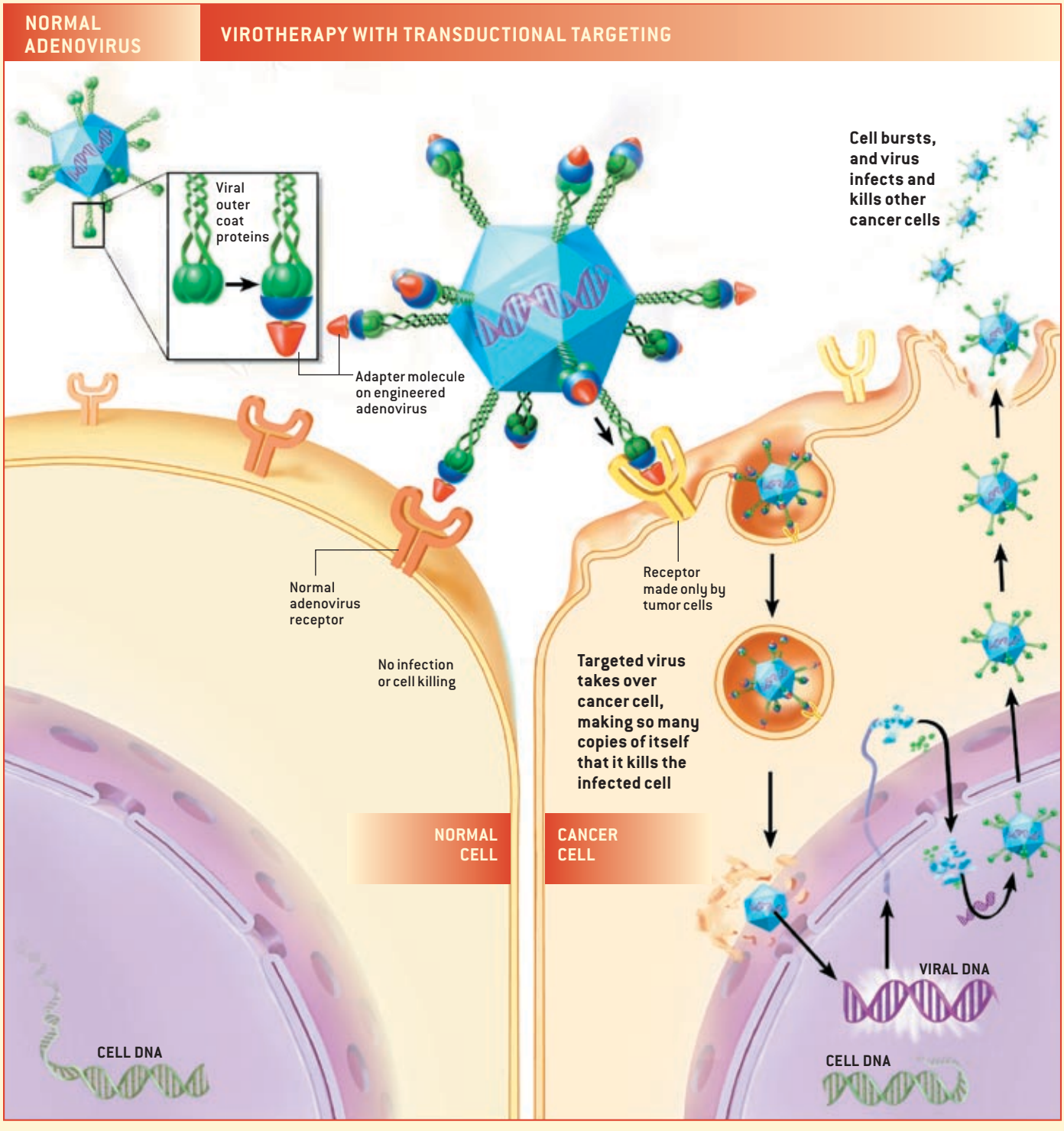
ZAPPING CANCER CELLS WITH VIRUSES

Two main strategies are being explored for virotherapy, which is the technique of using reproducing viruses to kill tumors. In the first method, dubbed transductional targeting (*below*), scientists are attempting to engineer viruses such as adenoviruses—which normally cause respiratory infections—to selectively infect and destroy only cells that have turned cancerous. They are attaching adapter molecules onto the viral outer coat proteins or directly modifying these proteins to try to prevent the viruses from entering normal cells and to instead prompt them to home in on tumor cells.


NORMAL ADENOVIRUS
VIROTHERAPY WITH TRANSDUCTIONAL TARGETING
Viral outer coat proteins
Adapter molecule on engineered adenovirus
Cell bursts, and virus infects and kills other cancer cells
Normal adenovirus receptor
No infection or cell killing
Receptor made only by tumor cells
Targeted virus takes over cancer cell, making so many copies of itself that it kills the infected cell
NORMAL CELL
CANCER CELL
CELL DNA
VIRAL DNA
CELL DNA

TERESE WINSLOW

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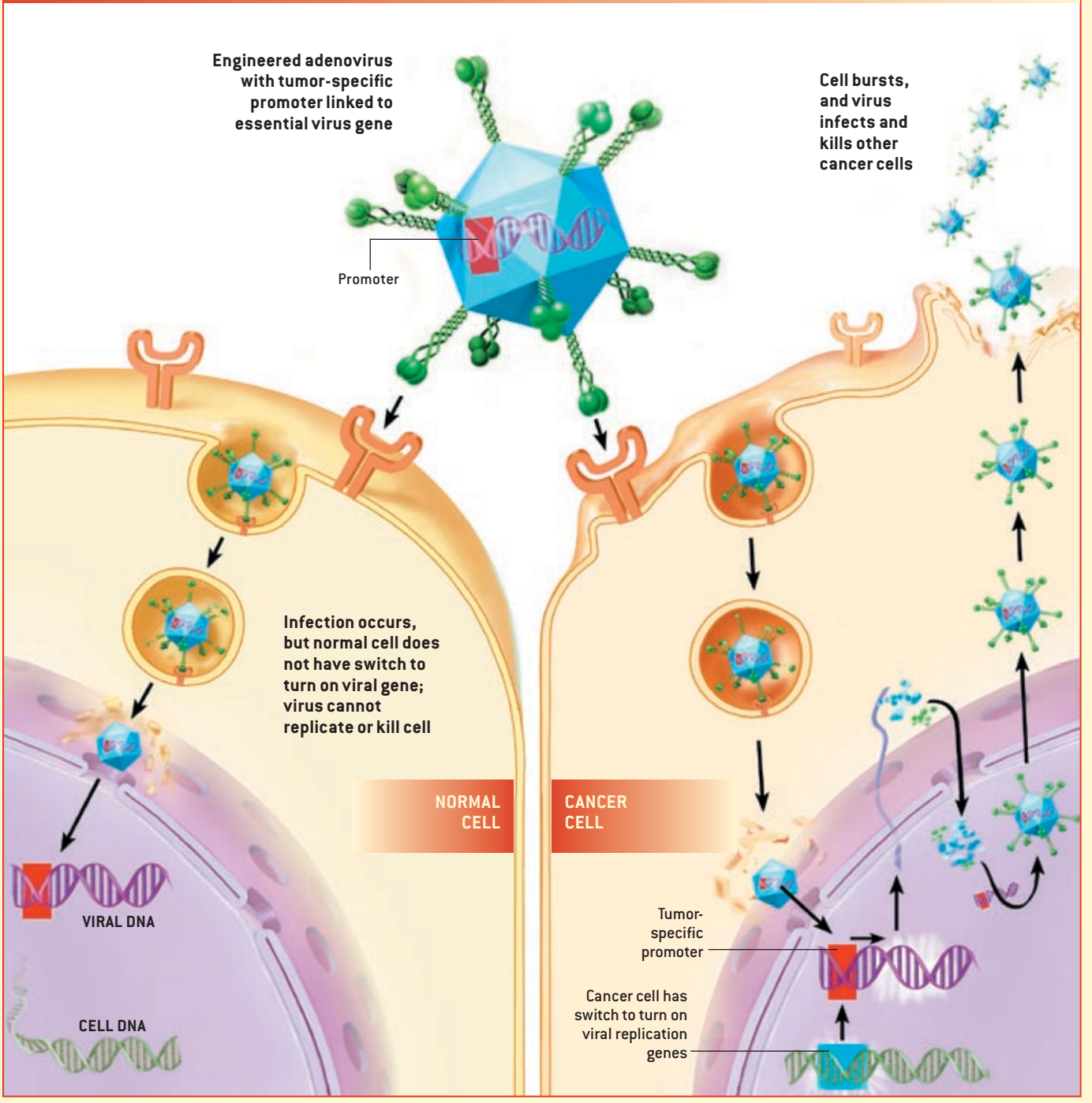
The second approach (*below*) involves placing a snippet of DNA called a tumor-specific promoter next to one of the adenovirus's essential genes. The promoter acts as an "on" switch that permits the gene to function only in cancer cells. The engineered viruses can enter normal cells, but they cannot reproduce and kill them. Once they enter cancer cells, however, the tumor-specific promoter lets them make thousands of copies of themselves and ultimately burst the cancer cells. They can then spread to—and destroy—other tumors. *—D.M.N., R.D.A. and D.T.C.*

VIROTHERAPY WITH TRANSCRIPTIONAL TARGETING


Engineered adenovirus with tumor-specific promoter linked to essential virus gene
Promoter
Cell bursts, and virus infects and kills other cancer cells
Infection occurs, but normal cell does not have switch to turn on viral gene; virus cannot replicate or kill cell
NORMAL CELL
CANCER CELL
VIRAL DNA
CELL DNA
Tumor-specific promoter
Cancer cell has switch to turn on viral replication genes

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cer Foundation, have tested the approach in men whose prostate cancer recurred following treatment with radiation. The researchers used adenoviruses that had been engineered by Cell Genesys to contain the promoter for prostate-specific antigen, a protein made in abundance by prostate tumors. They administered the virotherapy to 20 men who received varying doses of the adenoviruses. In 2001 Simons and his colleagues reported that none of the men experienced serious side effects and that the tumors of the five men who received the highest doses of the virotherapy shrank by at least 50 percent.

Other Strategies

VIROTHERAPISTS might end up combining the transductional and transcriptional targeting strategies to ensure that the viruses kill only tumor cells and not normal ones. Adenoviruses engineered to contain the promoter for the enzyme that makes melanin, for instance, can also replicate in normal melanocytes, so on their own they might cause spots of depigmentation. And adenoviruses that are designed to bind to receptors on the surfaces of tumor cells can still invade a small proportion of healthy cells. But viruses altered to have several fail-safe mechanisms would be expected to be less likely to harm normal cells. There are no results at present, however, to demonstrate that a combination of approaches makes viruses more targeted.

A further strategy for targeting virotherapy makes the most of one of cancer's hallmarks: the ability of tumor cells to divide again and again in an uncontrolled manner. Healthy cells make proteins that serve as natural brakes on cell division—notably the retinoblastoma (Rb) protein. As cells turn cancerous, however, the genes that code for one or the other of these proteins become mutated or otherwise inactivated. Certain viruses, including adenoviruses, interfere with the braking mechanisms of a normal cell by making proteins that stick to and inactivate Rb. They do this because they can replicate only in cells that are preparing to divide.

Several research groups and biotechnology companies have engineered adenoviruses that fail to make the Rb blockers. Normal cells, which make these blockers, will stall the replication of these viruses by putting the brakes on cell division. But these viruses will replicate in cells in which the Rb protein is already disabled—cancer cells—and kill them. Curiel and Alvarez are currently conducting a clinical trial of this approach using a virus that is also integrin-targeted, as described above. The first phase I study began in early 2007. In this trial, escalating dosages of a modified adenovirus are injected daily for three days into the abdomens of patients with recurrent ovarian or other gynecological cancers. The objectives are to determine the maximum dose that can be tolerated, the toxicity,

But Is It Safe?

Many approaches to virotherapy use adenoviruses, which caused a death in a gene therapy clinical trial

Jesse Gelsinger, who died in 1999 after receiving an infusion of adenoviruses, in a family photograph.

In September 1999 18-year-old Jesse Gelsinger died after receiving an infusion of adenoviruses into his liver. He had a mild form of an inherited liver disease called ornithine transcarbamylase deficiency (OTCD) and was participating in a clinical trial of a new gene therapy to use adenoviruses to ferry a corrected copy of the gene encoding OTCD into his liver cells. Unfortunately, four days after an infusion of the viruses, he died of acute respiratory distress syndrome and multiple organ failure, apparently caused by an overwhelming immune reaction to the large dose of adenoviruses he had been administered as part of the trial.

Although Gelsinger's death was part of a gene therapy trial, the tragedy also has ramifications for the young field of virotherapy. Gene therapy uses crippled versions of viruses such as adenoviruses to introduce a new gene into cells; virotherapy employs actively replicating viruses (which may or may not contain added genes), including adenoviruses, to kill specific types of cells.

Gelsinger's autopsy showed that the engineered adenoviruses had spread to his spleen, lymph nodes and bone marrow, and an examination of his records revealed that his liver function was probably too impaired for him to be a volunteer in the trial. A number of scientists have also suggested that he might have mounted such an extreme immune reaction because he had previously been infected with a naturally occurring adenovirus.

Since Gelsinger's death, gene therapists and virotherapists alike have focused on refining adenoviruses to make them safer. But researchers are still unsure why Gelsinger reacted so violently to the adenoviral infusions: a second patient participating in the same clinical trial tolerated a similar dose of the viruses. And hundreds of other people worldwide have been treated so far with adenoviruses, including replication-competent adenoviruses, with no serious side effects.

A National Institutes of Health report generated in the aftermath of Gelsinger's demise recommends that all participants in such clinical trials be monitored closely for toxic reactions before and after the infusion of therapeutic viruses. It also stipulates that volunteers be screened for any predisposing conditions that would increase their sensitivity for the viruses.

—D.M.N., R.D.A. and D.T.C.

MBR/NEWSCOM

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Selected Companies Involved in Virotherapy

COMPANY	HEADQUARTERS	VIRUS	DISEASES	VIRAL MODIFICATIONS	CLINICAL TRIAL STATUS
BioVex	Abingdon, England	Herpes simplex virus (HSV)	Breast cancer and melanoma	Carries the gene for granulocyte-macrophage colony stimulating factor, an immune system stimulant	Phase I/II
Cell Genesys	South San Francisco, Calif.	Adenovirus	Prostate cancer	Targeted to prostate cancer cells using prostate-specific promoters	Phase I/II
Crusade Laboratories	Glasgow, Scotland	HSV	Glioma (brain cancer), head and neck cancer, melanoma	Has a gene deletion that restricts it to actively dividing cells such as cancers	Phase II for glioma and head and neck cancer; phase I for melanoma
MediGene	Martinsried, Germany	HSV	Glioma and colon cancer that has spread (metastasized)	Harbors two gene deletions that prevent it from reproducing in normal cells	Phase II for glioma; phase I for colon cancer metastases
Oncolytics Biotech	Calgary, Alberta	Reovirus	Prostate cancer and glioma	Able to replicate only in cancer cells bearing the activated oncogene *RAS*	Phase II for prostate cancer; phase I/II for glioma
VectorLogics	Birmingham, Ala.	Adenovirus	Ovarian cancer	Expanded tropism for cancer cells and reduced replication in normal cells	Phase I/II

NOTE: Phase I tests are designed to evaluate safety in small numbers of patients. Phases II and III are intended to determine the appropriate dose and efficacy, respectively.

the immunological response, and any antitumor effects of the adenovirus. To date, nine patients have been treated with dosages of up to 10 billion virus particles a day. No toxicity has been detected, and more patients are joining the trial.

Researchers are also arming therapeutic viruses with genes that make the cells they infect uniquely susceptible to chemotherapy. The technique involves splicing into the viruses genes that encode enzymes that turn nontoxic precursors, or "prodrugs," into noxious chemotherapies. In one example, which was reported in 2002, André Lieber of the University of Washington and his co-workers designed adenoviruses to carry genes encoding the enzymes capable of converting innocuous prodrugs into the anticancer compounds camptothecin and 5-fluorouracil. The scientists engineered the viruses so that they could make the enzymes only in actively dividing cells, such as cancer cells. When they injected the viruses and the prodrugs into mice bearing implanted human colon or cervical cancer cells, they found that the viruses reproduced and spread in the tumors.

Such "smart" virotherapies are the vanguard of the future. But physicians will also need to track the activity of virotherapies in a patient's body to best assess how well the strategies are working and to refine them further. Virotherapists are now teaming with radiologists to establish novel imaging technologies to easily measure how effectively a given virotherapy is replicating.

The imaging strategies involve inserting a gene that governs the production of a tracer molecule into a virus or virus-infected cell. The tracer can be either a fluorescent protein that can be observed directly or one that binds to, or activates and traps, the radionuclides used in standard radiological imaging techniques. The fluorescent protein might work best for cancers that are accessible by an endoscope, such as cancers of the larynx. Physicians could peer into the endoscope and see exactly where the viruses—and, therefore, cancer cells—are by looking for fluorescence. So far the approach has worked best with viruses that do not kill cells, however. Nevertheless, we are convinced that such sophisticated imaging technologies will enable scientists to draw more meaningful conclusions from future clinical trials of virotherapy.

In 1995 gene therapy pioneer W. French Anderson of the University of Southern California School of Medicine predicted in *Scientific American* that "by 2000 … early versions of injectable vectors that target specific cells will be in clinical trials." These trials indeed began on schedule, as well as some he could not have envisioned then. We envision a substantial role for viruses—that is, therapeutic viruses—in 21st-century medicine. SA

MORE TO EXPLORE

Gene Therapy: Designer Promoters for Tumour Targeting. D. M. Nettelbeck, V. Jérôme and R. Müller in *Trends in Genetics,* Vol. 16, No. 4, pages 174–181; April 2000.

Replicative Adenoviruses for Cancer Therapy. R. Alemany, C. Balagué and D. T. Curiel in *Nature Biotechnology,* Vol. 18, No. 7, pages 723–727; July 2000.

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Gene therapy clinical trials database of *Journal of Gene Medicine:* **www.wiley.com/legacy/wileychi/genmed/clinical/**

American Society of Gene Therapy: **www.asgt.org**

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New Light on Medicine

Pigments that turn caustic on exposure to light can fight cancer, blindness and heart disease. Their light-induced toxicity may also help explain the origin of vampire tales

Stories of vampires date back thousands of years.

Our modern concept stems from Bram Stoker's quirky classic *Dracula* and Hollywood's Bela Lugosi—the romantic, sexually charged, bloodsucking outcast with a fatal susceptibility to sunlight and an abhorrence of garlic and crosses. In contrast, vampires of folklore cut a pathetic figure and were also known as the undead. In searching for some underlying truth in vampire stories, researchers have speculated that the tales may have been inspired by real people who suffered from a rare blood disease, porphyria. And in seeking treatments for this disorder, scientists have stumbled on a new way to attack other, more common serious ills.

Porphyria is actually a collection of related diseases in which pigments called porphyrins accumulate in the skin, bones and teeth. Many porphyrins are benign in the dark but are transformed by sunlight into caustic, flesh-eating toxins. Without treatment, the worst forms of the disease (such as congenital erythropoietic porphyria) can be grotesque, ultimately exacting the kind of hideous disfigurement one might expect of the undead. The victims' ears and nose get eaten away. Their lips and gums erode to reveal red, fanglike teeth. Their skin acquires a patchwork of scars, dense pigmentation and deathly pale hues, reflecting underlying anemia. Because anemia can be treated with blood transfusions, some historians speculate that

By Nick Lane

LIGHT-ACTIVATED DRUGS used in photodynamic therapy could treat diseases of the eye, cancers such as those of the esophagus, and coronary artery disease.

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in the dark ages people with porphyria might have tried drinking blood as a folk remedy. Whatever the truth of this claim, those with congenital erythropoietic porphyria would certainly have learned not to venture outside during the day. They might have learned to avoid garlic, too, for some chemicals in garlic are thought to exacerbate the symptoms of the disease, turning a mild attack into an agonizing reaction.

While struggling to find a cure for porphyria, scientists came to realize that porphyrins could be not just a problem but a tool for medicine. If a porphyrin is injected into diseased tissue, such as a cancerous tumor, it can be activated by light to destroy that tissue. The procedure is known as photodynamic therapy, or PDT, and has grown from an improbable treatment for cancer in the 1970s to a sophisticated and effective weapon against a diverse array of malignancies today and, most recently, for macular degeneration and pathological myopia, common causes of adult blindness. Ongoing research includes pioneering treatments for coronary artery disease, AIDS, autoimmune diseases, transplantation rejection and leukemia.

Molecular Mechanisms

THE SUBSTANCES at the heart of porphyria and photodynamic therapy are among the oldest and most important of all biological molecules, because they orchestrate the two most critical energy-generating processes of life: photosynthesis and oxygen respiration. Porphyrins make up a large family of closely related compounds, a colorful set of evolutionary variations on a theme. All porphyrins have in common a flat ring (composed of carbon and nitrogen) with a central hole, which provides space for a metal ion such as iron or magnesium to bind to it. When aligned correctly in the grip of the porphyrin rings, these metal atoms catalyze the most fundamental energy-generating processes in biology. Chlorophyll, the plant pigment that absorbs the energy of sunlight in photosynthesis, is a porphyrin, as is heme, which is at the heart of the oxygen-transporter protein hemoglobin and of many enzymes vital for life, including cytochrome oxidase (which generates energy by transferring electrons to oxygen in a critical step of cellular respiration).

Porphyria arises because of a flaw in the body's heme-making machinery. The body produces heme and other porphyrins in a series of eight coordinated stages, each catalyzed by a separate enzyme. Iron is added at the end to make heme. In porphyria, one of the steps does not occur, leading to a backlog of the intermediate compounds produced earlier in the sequence. The body has not evolved to dispose of these intermediates efficiently, so it dumps them, often in the skin. The intermediates do not damage the skin directly, but many of them cause trouble indirectly. Metal-free porphyrins (as well as metalloporphyrins containing metals that do not interact with the porphyrin ring) can become excited when they absorb light at certain wavelengths; their electrons jump into higher-energy orbitals. The molecules can then transmit their excitation to other molecules having the right kind of bonds, especially oxygen, to produce reactive singlet oxygen and other highly reactive and destructive molecules known as free radicals. Metal-free porphyrins, in other words, are not the agents, but rather the brokers, of destruction. They catalyze the production of toxic forms of oxygen.

Photosensitive reactions are not necessarily harmful. Their beneficial effects have been known since ancient times. In particular, some seeds and fruits contain photosensitive chemicals (photosensitizers) called psoralens, which indirectly led scientists to experiment with porphyrins. Psoralens have been used to treat skin conditions in Egypt and India for several thousand years. They were first incorporated into modern medicine by Egyptian dermatologist Abdel Monem El Mofty of Cairo University some 60 years ago, when he began treating patients with vitiligo (a disease that leaves irregular patches of skin without pigment) and, later, those with psoriasis using purified psoralens and sunlight. When activated by light, psoralens react with DNA in proliferating cells to kill them.

Two American dermatologists, the late Aaron B. Lerner of Yale University and the late Thomas B. Fitzpatrick of Harvard University, were struck by the potential of psoralens. In the 1960s they showed that psoralens are activated by ultraviolet (UVA) rays, and the researchers later refined psoralen therapy using an ultraviolet lamp similar to those used in solaria today. Their method became known as PUVA (short for psoralen with UVA) and is now one of the most effective treatments for psoriasis and other skin conditions.

Overview/*Light Therapy*

- In photodynamic therapy, light-activated chemicals called porphyrins are used to destroy fast-growing cells and tissue. Doctors could apply the treatment to a variety of ailments, including age-related macular degeneration, tumors and atherosclerotic plaques.
- A few porphyrin drugs are on the market, and several others are undergoing human trials.
- Researchers got the idea for photodynamic therapy from their knowledge of the rare disease porphyria, in which porphyrins accumulate in the skin and certain organs. Unless the disease is managed, victims of the severest type of porphyria can become disfigured, leading some researchers to speculate that they may have inspired medieval vampire legends.

A Way to Kill Cancer Cells?

IN THE EARLY 1970S the success of PUVA impressed Thomas J. Dougherty of the Roswell Park Cancer Institute in Buffalo, N.Y., leading him to wonder if a variant of it could be effective against cancer. Activated psoralens can kill rogue cells to settle inflammation, but in comparison with porphyrins they are not potent photosensitizers. If psoralens could kill individual cells, could porphyrins perhaps devour whole tumors? His idea was the beginning of true photodynamic

PHOTOGRAPH BY SAM OGDEN (*preceding page*)

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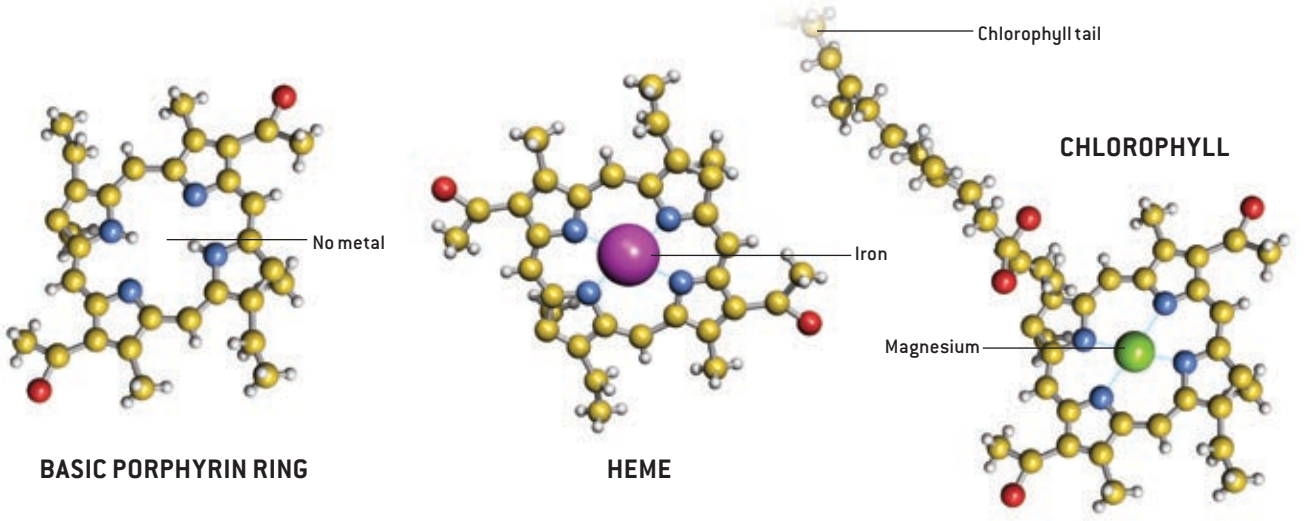

PORPHYRINS all have in common a flat ring, mainly composed of carbon and nitrogen, and a central hole where a metal ion can sit. The basic ring (*far left*) becomes caustic when exposed to light; molecules useful for photodynamic therapy also share this trait. Nontoxic examples include heme (a component of the oxygen transporter hemoglobin) and the chlorophyll that converts light to energy in plants.

therapy, in which photosensitizers catalyze the production of oxygen free radicals. The concept was built on pioneering work from German physicians Oscar Raab, then a medical student, and Hermann von Tappeiner, his professor. Around the start of the 20th century, Raab and von Tappeiner showed that acridine, when activated by light, reacts with oxygen to kill protozoa such as paramecia. Von Tappeiner had even gone on to treat patients with skin cancer using eosin, a photosensitive component of coal tar, and white light. Dougherty realized, however, that these early forms of photodynamic therapy lacked the raw power of porphyrins. He also drew on two other medically useful properties of porphyrins discovered in the mid-20th century: porphyrins accumulate selectively in cancer cells and are activated by red light, which penetrates more deeply into biological tissues than do shorter wavelengths, such as white light or UVA.

Dougherty injected a mixture of porphyrins into the bloodstream of mice with mammary tumors. He then waited a few days for the porphyrins to build up in the tumors before shining red light on them. His early setup was primitive, passing light from an old slide projector through a 35-millimeter slide colored red. His results were nonetheless spectacular. The light activated the porphyrins within the tumor, which transferred their energy to oxygen in cells to damage the surrounding tissues. In almost every case, the tumors blackened and died after the light treatment. There were no signs of recurrence.

Dougherty and his colleagues published their data in 1975 in the *Journal of the National Cancer Institute*, with the brave title "Photoradiation Therapy II: Cure of Animal Tumors with Hematoporphyrin and Light." Over the next few years they refined their technique by using a low-power laser to focus red light onto the tumors. They went on to treat more than 100 patients in this way, including people with cancers of the breast, lung, prostate and skin. Their outcomes were gratifying, with a "complete or partial response" in 111 of 113 tumors.

Sadly, though, cancer is not so easily beaten. As more physicians started trying their hand with PDT, some serious drawbacks began to emerge. The affinity of porphyrins for tumors turned out to be a bit of an illusion—porphyrins are taken up by any rapidly proliferating tissue, including the skin, leading to photosensitivity. Although Dougherty's original patients were no doubt careful to avoid the sun, nearly 40 percent of them reported burns and skin rashes in the weeks after PDT.

Potency was another issue. The early porphyrin preparations were mixtures, and they were seldom strong enough to kill the entire tumor. Some porphyrins are not efficient at passing energy to oxygen; others are activated only by light that cannot penetrate more than a few millimeters into the tumor. Some biological pigments normally present in tissues, such as hemoglobin and melanin, also absorb light and in doing so can prevent a porphyrin from being activated. Even the porphyrin itself can cause this problem if it accumulates to such high levels that it absorbs all the light in the superficial layers of the tumor, thus preventing penetration into the deeper layers.

Many of these difficulties could not be resolved without the help of specialists from other disciplines. Chemists were needed to create new, synthetic porphyrins, ones that had greater selectivity for tumors and greater potency and that would be activated by wavelengths of light able to reach farther into tissues and tumors. (For each porphyrin, light activation and absorption occur only at particular wavelengths, so the trick is to design a porphyrin that has its absorption maximum at a wavelength that penetrates into biological tissues.) Physicists were needed to design sources that could produce light of particular wavelengths to activate the new porphyrins or that could be attached to fine endoscopes and catheters or even implanted in tissues. Pharmacologists were needed to devise ways of reducing the time that porphyrins spent circulating in the bloodstream, thereby restricting photosensitive side effects. Finally, clinicians were needed to design trials that could

THE AUTHOR

NICK LANE studied biochemistry at Imperial College, University of London. His doctoral research, at the Royal Free Hospital, concentrated on oxygen free radicals and metabolic function in organ transplants. Lane is an honorary reader at University College London and a regular contributor to *Nature*. His books include *Oxygen: The Molecule That Made the World* and *Power, Sex, Suicide: Mitochondria and the Meaning of Life*.

SLIM FILMS

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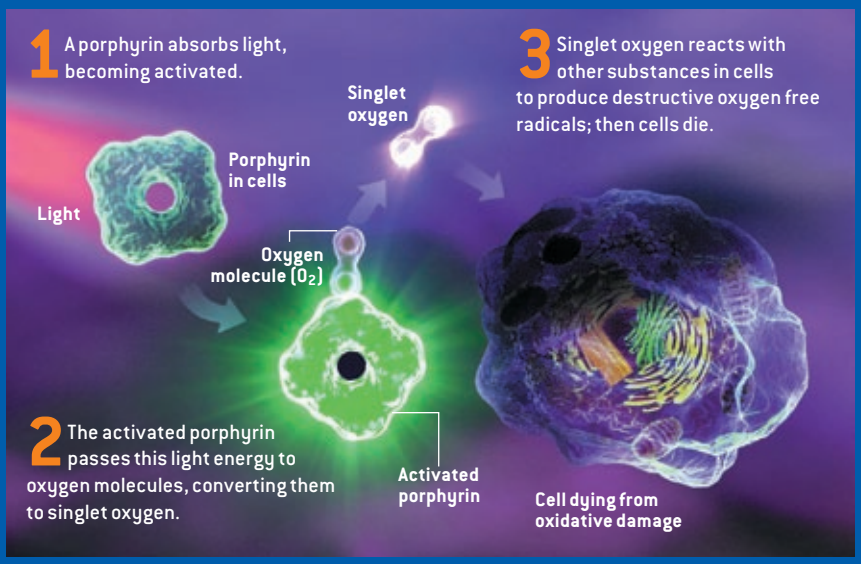
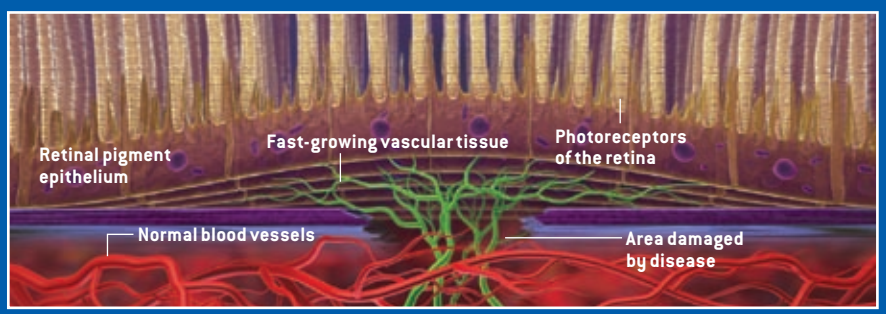
HOW PHOTODYNAMIC THERAPY WORKS

Doctors who administer photodynamic therapy deliver photosensitive chemicals called porphyrins intravenously. These chemicals then collect in rapidly proliferating cells and, when exposed to light, initiate a cascade of molecular reactions that can destroy those cells and the tissues they compose. Some targets for the therapy include abnormal blood vessels in the retinas of people with age-related macular degeneration (the leading cause of adult blindness), cancerous tumors, and atherosclerotic plaques in coronary arteries.

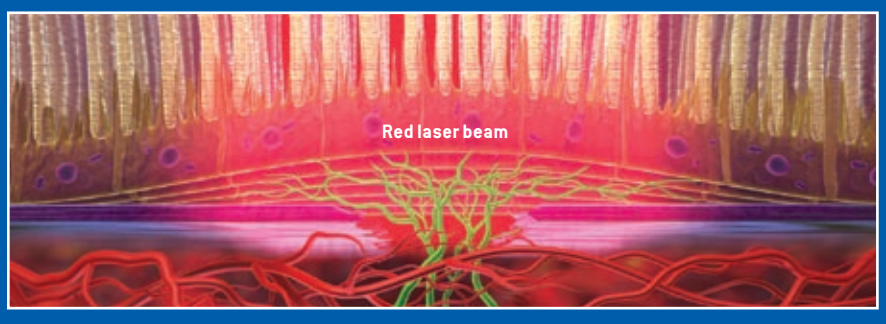
. . . AT THE MOLECULAR LEVEL


1 A porphyrin absorbs light, becoming activated.
Light
Porphyrin in cells
Singlet oxygen
3 Singlet oxygen reacts with other substances in cells to produce destructive oxygen free radicals; then cells die.
Oxygen molecule (O_2)
2 The activated porphyrin passes this light energy to oxygen molecules, converting them to singlet oxygen.
Activated porphyrin
Cell dying from oxidative damage

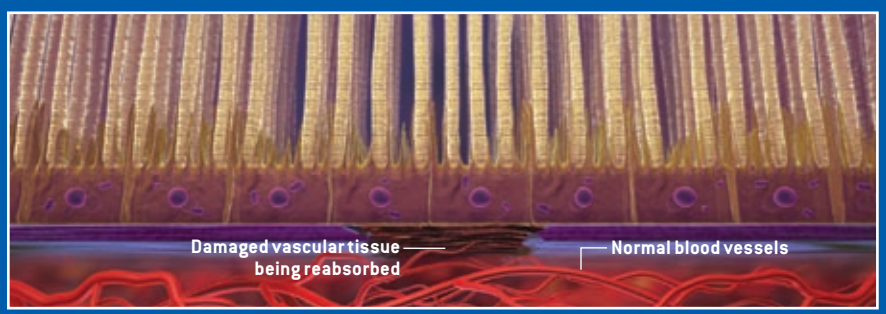
. . . IN THE EYE



1 To treat macular degeneration, a porphyrin (*green*) is injected into a patient's arm. It takes just 15 minutes for the porphyrin to accumulate in abnormal blood vessels under the macula, the central part of the retina responsible for color vision.



2 A red laser light activates the porphyrin, which leads to the destruction of the vascular tissue.



3 After therapy halts damage to the retina, the treated vascular tissue is reabsorbed by the body, and the overlying photoreceptors may settle back into place. Because vessel growth could recur, the patient may require several additional treatments.

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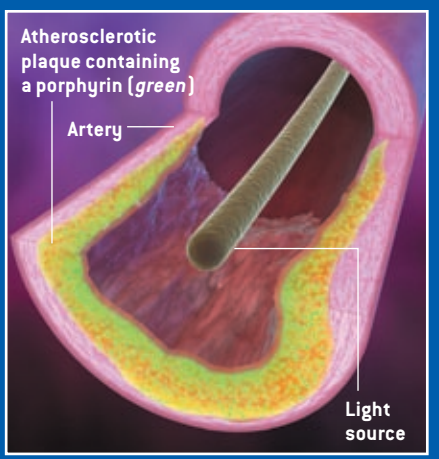
... DEEP IN THE BODY

Even long wavelengths of visible light cannot penetrate very far into tissue, so photodynamic therapies for diseased tissue deep within the body require an internal light-delivery system.

1 Here, in an experimental therapy, an optical fiber has been threaded into an artery in which a porphyrin has accumulated in atherosclerotic plaques.



2 The fiber produces red light, activating the porphyrin.

3 Over the course of a few days, the porphyrin destroys unwanted plaques.

HYBRID MEDICAL ANIMATION

prove an effect and determine the best treatment regimens.

The ideal drug would be not only potent and highly selective for tumors but also broken down quickly into harmless compounds and excreted from the body. The first commercial preparation, porfimer sodium (Photofrin), was approved by the U.S. Food and Drug Administration for the treatment of various cancers. Although it has been helpful against certain cancers (including esophageal, bladder, head and neck, and skin cancers and some stages of lung cancer), it has not been the breakthrough that had been hoped for and cannot yet be considered an integral part of cancer therapy. Surprisingly, though, the first photosensitizing drug to fulfill most of the stringent criteria for potency and efficacy without causing photosensitivity, verteporfin (Visudyne), was approved in April 2000 by the FDA not to treat cancer at all but to prevent blindness. As the theories converged with reality, researchers came to realize that PDT can do far more than destroy tumors.

Battling Blindness

ONE THING IT could do, for instance, was combat age-related macular degeneration (AMD), the most common cause of legal blindness in our maturing Western population. Most people who acquire AMD have a benign form and do not lose their sight, but about a tenth have a much more aggressive type called wet AMD. In this case, abnormal, leaking blood vessels, like miniature knots of varicose veins, grow underneath the retina and ultimately damage the sharp central vision required for reading and driving. As the disease progresses, central vision is obliterated, making it impossible to recognize people's faces or the details of objects.

Most attempts to hinder this grimly inexorable process have failed. Dietary antioxidants may be able to delay the onset of the disorder but have little effect on the progression of established disease. Until recently, the only treatment proved to slow the progression of wet AMD was a technique called laser photocoagulation. The procedure involves applying a thermal laser to the blood vessels to fuse them and thus halt their growth. Unfortunately, the laser also burns the normal retina and so destroys a small region to prevent later loss of vision in the rest of the eye. Whether this is worth it depends on the area of the retina that needs to be treated. For most people diagnosed with wet AMD, the area is located below the critical central part of vision or is already too large to benefit from laser coagulation.

Against this depressing backdrop, researchers at Harvard and at the biotechnology firm QLT in Vancouver, B.C., reasoned that PDT might halt the growth of these blood vessels and delay or even prevent blindness. If porphyrins could accumulate in any rapidly proliferating tissue—the very problem in cancer—then perhaps they could also accumulate in the blood vessels growing under the retina. Verteporfin, a novel synthetic porphyrin, seemed promising because it had a good track record in preclinical animal studies at QLT and at the University of British Columbia in the late 1980s and early 1990s.

Verteporfin accumulates in abnormal retinal vessels re-

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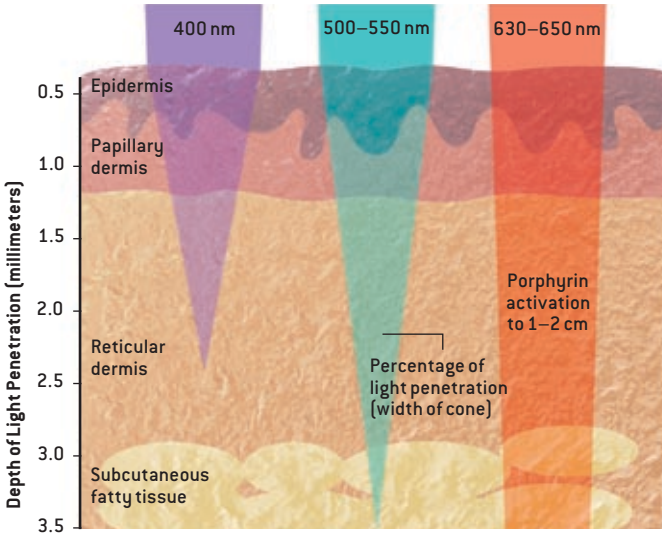

EACH WAVELENGTH OF LIGHT reaches a different depth in tissues, and any given porphyrin absorbs light at specific wavelengths. A porphyrin activated by deeper-penetrating light might be best for treating an internal tumor. In contrast to porphyrins, the psoralens used in PUVA treatments for psoriasis are activated by near-ultraviolet light (400 nanometers), which barely penetrates the skin.

markably quickly: within 15 minutes of injection into an arm vein. When activated by red laser light, verteporfin seals off the vessels, sparing the overlying retina. Any blood vessels that grow back can be nipped in the bud by further treatments. Two major clinical trials, headed by Neil M. Bressler of the Wilmer Eye Institute at Johns Hopkins University, confirmed that PDT can be given six or seven times over a three-year period without damaging a healthy retina. For people with the most aggressive form of AMD (with mostly "classic" lesions), verteporfin halved the risk of moderate or serious vision loss over a two-year period. The effect is sustained over at least three or four years: patients who are not treated lose as much vision in three months as those treated with verteporfin lose in three years.

Some participants in the trials gained little benefit from PDT. Their disease may have already progressed too far. A reanalysis of clinical data presented by Bressler in April 2002 at the International Congress of Ophthalmology in Sydney showed that smaller lesions respond much better to treatment than older, larger ones, implying that early detection and treatment may optimize the benefits of PDT.

Other Treatment Avenues

THE SUCCESS OF ophthalmic PDT has inspired research activity in other fields but also reveals the drawbacks of the treatment. In particular, even red light penetrates no more than a few centimeters into biological tissues [*see illustration above*]. This limitation threatens the utility of PDT in internal medicine—its significance might seem to be skin deep. There are ways of turning PDT inward, however. One ingenious idea is called photoangioplasty, which is now being tested to treat coronary artery disease.

Coronary angioplasty is a minimally invasive procedure for treating arteries affected by atherosclerosis. It uses a tiny balloon to open arteries, so that atherosclerotic plaques do not occlude the entire vessel. Photoangioplasty could sidestep many of the problems of conventional angioplasty, notably the restenosis (renarrowing) of treated arteries. The procedure involves injecting a porphyrin into the bloodstream, waiting for it to build up in the damaged arterial walls and then illuminating the artery from the inside, using a tiny light source attached to the end of a catheter. The light activates the porphyrins in the plaques, destroying the abnormal tissues while sparing the normal walls of the artery.

An even more ingenious method for treating deep cancers is the use of self-lighting nanoparticles. Wei Chen of the University of Texas at Arlington recently developed a method of attaching porphyrins to "scintillation luminescence nanoparticles." The nanoparticles generate visible light when exposed to x-rays during radiotherapy, and this in turn activates the porphyrin. The method effectively combines radiotherapy with PDT, optimizing both methods and allowing the treatment of large or deeply buried tumors with relatively low-dose radiotherapy. But how will the bulky nanoparticles be targeted at tumor cells? In January, Chen and his colleagues reported preliminary findings suggesting that the entire complex can be attached to folic acid, which is then taken up by folate receptors on cancer cells.

An alternative approach for targeting porphyrins or even nanoparticle complexes at tumors involves attaching them to antibody fragments that recognize cancer cells. Mahendra Deonarain and his colleagues at Imperial College London, who are pioneers of this method, have managed to attach more than 10 porphyrin molecules to a single antibody fragment without destroying its ability to target cancer cells. The group used antibody fragments rather than whole antibodies to reduce the size of the complex and enable it to be cleared from the body more quickly. The team's initial findings, reported in March, showed the complexes accumulating in tumors at 10 times their concentration in blood and 50 times their concentration in muscle.

Accumulation of porphyrins in active and proliferating cells raises the possibility of treating other conditions in which abnormal cell activation or proliferation plays a role—among them, infectious diseases. Attempts to treat infections with the pigments had long been frustrated by a limited effect on gram-negative bacteria, which have a complex cell wall that obstructs the uptake of porphyrins into these organisms. One solution, developed by Michael R. Hamblin and his colleagues at Harvard, involved attaching a polymer—usually polylysine, a repetitive chain of the amino acid lysine—to the porphyrin. The polymer disrupts the lipid structure of the bacterial cell wall, enabling the porphyrins to gain entry to the cell. Once inside, they can be activated by light to kill the bacteria. In studies of animals with oral infections and infected wounds, the altered porphyrin showed potent antimicrobial activity against a broad spectrum of gram-negative and gram-positive

HYBRID MEDICAL ANIMATION

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Photodynamic Therapies

The light-activated drugs listed below are a sampling of those on the market or in development.

DRUG	TARGET	MAKER	STATUS
LEVULAN (5-aminolevulinic acid)	Acne and actinic keratosis (precancerous skin disorder), Barrett's esophagus (precancerous condition)	DUSA PHARMACEUTICALS Toronto	On the market for actinic keratosis; phase II trials (relatively small studies in humans) have begun for acne
PHOTOFRIN (porfimer sodium)	Cancers of the esophagus and lung, high-grade dysplasia from Barrett's esophagus	AXCAN SCANDIPHARM Birmingham, Ala.	On the market for esophageal cancer and nonsmall cell lung cancer; FDA-approved for high-grade dysplasia
VISUDYNE (verteporfin)	Age-related macular degeneration, pathological myopia and ocular histoplasmosis (eye disorders)	QLT and NOVARTIS OPHTHALMICS Vancouver, B.C., and Duluth, Ga.	On the market
METVIX (methylaminolevulinic acid)	Actinic keratosis, basal cell skin cancer and squamous cell skin cancer	PHOTOCURE Oslo, Norway	FDA-approved for actinic keratosis and basal cell skin cancer
LS11 (talaporfin sodium)	Colorectal cancer, hepatocellular carcinoma, and glioma	LIGHT SCIENCES ONCOLOGY Bellevue, Wash.	In phase III trials for colorectal cancer with recurrent liver metastases and for hepatocellular carcinoma; phase II trial for glioma completed in February
TOOKAD (vascular-targeted PDT)	Prostate cancer	STEBA BIOTECH Toussus-le-Noble, France	In phase II/III trials for prostate cancer after radiation therapy

bacteria. As antibiotic resistance becomes more intractable, targeted antimicrobial PDT could become a useful weapon in the medical arsenal.

Several other, related photodynamic methods hinge on the finding that activated immune cells take up greater amounts of photosensitizing drugs than do quiescent immune cells and red blood cells, sparing the quiet cells from irreversible damage. In most infections, nobody would wish to destroy activated immune cells: they are, after all, responsible for the body's riposte to the infection. In these cases, targeting immune cells would be equivalent to "friendly fire" and would give the infection free rein to pillage the body.

In AIDS, however, the reverse is true. The AIDS virus, HIV, infects the immune cells themselves. Targeting infected immune cells would then be more like eliminating double agents. In the laboratory, HIV-infected immune cells take up porphyrins, thereby becoming vulnerable to light treatment. In patients, the light could be applied either by withdrawing blood, illuminating it and transfusing it back into the body (extracorporeal phototherapy) or by shining red light onto the skin, in what is called transdermal phototherapy. In the transdermal approach, light would eliminate activated immune cells in the circulation as they passed through the skin. Whether the technique will be potent enough to eliminate diseased immune cells in HIV-infected patients remains an open question.

Autoimmune diseases, rejection of organ transplants, and leukemias are also all linked by the common thread of activated and proliferating immune cells. In autoimmune diseases, components of our own body erroneously activate immune cells. These activated clones then proliferate in an effort to destroy the perceived threat—say, the myelin sheath in multiple sclerosis or the collagen in rheumatoid arthritis. When organs are implanted, activated immune cells may multiply to reject the foreign tissue—the transplanted organ or even the body tissues of the new host, in the case of bone marrow transplants. In leukemia, immune cells and their precursors in the bone marrow produce large numbers of nonfunctional cells. In each instance, PDT could potentially eliminate the unwanted immune cells, while preserving the quiescent cells, to maintain a normal immune response to infection. As in HIV infection, the procedure might work either extracorporeally or transdermally. Much of this research is in late-stage preclinical or early clinical trials. For all the cleverness in exploring possible medical applications, though, we can only hope that more extensive clinical studies will bear fruit. SA

MORE TO EXPLORE

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Gaining Ground on Breast Cancer

The newest targeted therapies are helping doctors to tailor increasingly effective treatments to individual patients

BY FRANCISCO J. ESTEVA AND GABRIEL N. HORTOBAGYI

Breast cancer is the most commonly diagnosed malignancy among women and, after lung cancer, the second leading cause of cancer-related deaths in North America. Yet unlike the survival rate for individuals diagnosed with lung cancer, the rate for women diagnosed with breast cancer has been rising dramatically over the past decade—to the point where breast cancer could soon lose its ranking as the second-greatest cancer killer. Nothing would delight clinicians like us more.

This improvement in overall outlook for women diagnosed with breast cancer is attributable in part to earlier detection, which results from greater awareness of, and access to, regular breast screening. But breast cancer patients are also benefiting from accelerated research that has led to a much better understanding of the disease and a wider variety of treatment choices that doctors can mix and match to tailor therapy for a particular patient. In just the past decade, it has even become possible to target drugs to specific molecules within tumors that help to drive the disease.

Breast cancer was, in fact, the first type of solid-tumor cancer to be treated with this molecular-targeting therapeutic approach, when the drug trastuzumab (Herceptin) was approved in 1998. The protein that trastuzumab was designed to attack, called HER2, promotes aggressive tumor growth. Before trastuzumab, diagnosis with a tumor that overproduces HER2 was dreaded news for patients. Now it can be one of the tumor types with the best prognosis, because doctors have an increasing number of effective weapons against HER2.

The next decade promises to be an exciting and productive time in the field of molecular-targeted cancer therapy: additional drugs currently being tested in people and animals are making it possible to go after an increasing variety of molecular tumor features that play a critical role in the initiation and survival of malignancies and in the cancers' progression to increasingly threatening stages. Along with improvements in older therapies and supportive care, this newer generation of drugs gives doctors more options for customizing treatment to cope with a tumor's particular suite of molecular characteristics and reflects our growing realization that breast cancer is not a single disease.

Overview/*Targeted Treatment*

- Breast cancer survival rates have been steadily climbing in North America and Europe, thanks to increased early detection and novel treatment options.
- Many new treatments target specific molecules on tumor cells, allowing doctors to tailor medication to an individual patient's tumor profile.
- Breast cancer was the first solid-tumor type for which molecular-targeted therapy became available, and the success of the approach promises further dramatic advances.

Evolving Treatment Approaches

ALTHOUGH THE PROSPECT of tailoring treatment to the molecular features of individual tumors is incredibly encouraging, prior advances are also contributing to the declining mortality rate for women diagnosed with breast cancer. Improved screening techniques, for instance, are definitely helping to catch and confirm more cases at an earlier stage, which is a boon, because breast cancer is highly curable if detected early. Newer imaging methods include digital mammography (which produces a clearer picture than screen-film mammography), ultrasound and magnetic resonance imaging (MRI).

In addition, surgical approaches to tumor excision have changed over the past 20 years from radical tissue removal in women whose tumor appears confined to a small part of the breast to breast-conserv-

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FAST FACTS

Inherited mutations in the *BRCA1* gene can multiply lifetime breast cancer risk by 10 times, but only in the past year have researchers discovered why. *BRCA1* is involved in DNA repair, so its malfunction makes errors in other cancer-promoting genes more likely.

Following a 2002 report that hormone replacement therapy (HRT) increased breast cancer risk in postmenopausal women, HRT use fell. The next year there was a dramatic drop in the incidence of both invasive (7.3 percent) and noninvasive (5.5 percent) breast cancers in the U.S.

ing therapy. More focused radiation is also less damaging to normal tissues such as those of the heart and lungs. These changes have made treatment less destructive, with equally successful results.

Besides these refinements in the detection and local management of breast tumors, the use of systemic therapies as supplementary, or adjuvant, treatments has become more sophisticated thanks to the availability of new drugs, improvements in their delivery, and management of side effects. Such treatments aim to eradicate any malignant cells not eliminated by surgery or radiation. The approach is often warranted because even tumors that are tiny and apparently self-contained can already have quietly spawned microscopic metastases, undetectable tumors at distant sites in the body. By attacking these invisible tumors, adjuvant chemotherapy can prolong disease-free intervals and overall survival rates.

Adjuvant chemotherapy is also improving the odds for those with more advanced tumors. In the 1970s our clinical group and others began developing multidisciplinary treatment programs for patients with so-called locally advanced breast cancer, which has invaded neighboring tissue. Such patients are often not diagnosed until their cancer is incurable with surgery alone. Our approach is to treat them with preoperative, or neoadjuvant, chemotherapy to shrink their tumors to operable size, after which surgery is performed, followed by additional chemotherapy and radiation therapy. Using this sandwich approach over the past three decades, specialized teams of doctors, nurses and other health professionals have greatly enhanced the cure rate for these patients. Even those whose breast tumor has already metastasized to other organs now have access to novel therapies that prolong their survival and to supportive care that increases their quality of life.

Another mainstay of breast cancer treatment, at least for patients with tumors that are determined to be dependent on estrogen or progesterone, is endocrine therapy. Indeed, hormonal manipulations to treat breast cancer date as far back as the 1890s, when doctors observed tumors regressing after they had removed the ovaries of premenopausal women with advanced breast disease. In 1966 researchers identified hormone receptors—molecules that bind to specific hormones—in various tissues, including that of the breast. Subsequent studies showed that a significant number of invasive breast cancers—as many as 75 percent—contain estrogen receptors or progesterone receptors, or both, causing these molecules to quickly become therapeutic targets.

The antiestrogen drug tamoxifen was first approved in the U.S. in 1977 to treat advanced breast cancer in postmenopausal women. The drug molecule binds to the estrogen receptor, preventing estrogen from doing so. Tamoxifen has since proved effective for patients with localized breast tumors that display estrogen or progesterone receptors and as a preventive therapy in healthy women who are at high risk for breast cancer. Meanwhile newer drugs that inhibit the aromatase enzyme, suppressing natural estrogen manufacture in the body, have proved superior to tamoxifen in postmenopausal women.

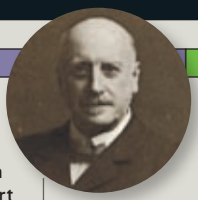
In a sense, then, estrogen and progesterone receptors became the first molecular features of tumors that could be directly targeted by drugs, although an important distinction should be noted between these targets and newer ones identified in the past decade. The sex steroid receptors promote cell proliferation, or growth, in healthy tissues as well as in tumors, so suppressing their ability to transmit growth signals does help to check tumor

RAISING AWARENESS of the importance of early detection, as well as raising funding for research, has paid off in notable declines in breast cancer mortality in the developed world.

TREATMENT MILESTONES

Doctors started aggressively treating breast cancer in the 19th century, with the first mastectomy done in 1882. But insights into mechanisms driving the disease that would lead to increasingly targeted therapies began with discoveries in the 1950s.

1880s–1890s



George T. Beatson

Hormonal cancer connection suggested when physicians report significant regression of breast cancer tumors following ovary removal or onset of menopause.

1896: First ovary removal performed as a breast cancer treatment by Beatson.

ANGELA ROWLINGS *AP Photo (racers)*; LISA APFELBACHER *(all pills)*; TIMELINE: WELLCOME LIBRARY, LONDON *(Beatson)*; MARK J. WINTER *Photo Researchers, Inc. (estrogen receptor)*; SCIENCE MUSEUM *(tamoxifen)*; P. MARAZZI *Photo Researchers, Inc. (Herceptin)*

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1950s–1960s

1951: Estrogen and testosterone found to drive the growth of breast and prostate cancers, respectively.

1958: Cancer researchers identify additional "growth factor" proteins that help tumors thrive.

1966: Estrogen receptor identified.

Estrogen receptor protein structure

1970s–1980s

Tamoxifen, a selective estrogen receptor modulator

1976: Cancer-promoting "oncogenes" first discovered in mammals.

1976: Clinical trials begin to show that lumpectomy with radiation can be as effective as mastectomy.

1977: Hormone-blocking drug tamoxifen approved in U.S. for treatment of breast cancers sensitive to estrogen or progesterone.

1988: Trial results show that preoperative chemotherapy shrinks tumors, making less invasive surgeries possible.

1990s–2008

1994: *BRCA1* gene, known to increase susceptibility to breast cancer, is isolated.

1998: Trastuzumab, the first molecular-targeted cancer therapy, approved in U.S. for use in breast cancer.

2007: Lapatinib, an inhibitor of growth signaling, approved in U.S. for use in breast cancer.

2008: Bevacizumab, an inhibitor of blood vessel formation in tumors, approved in U.S. for use in breast cancer.

Trastuzumab, also known by the trade name Herceptin

enlargement. And changes to the receptors' shape or function may sometimes contribute to the general malignant characteristics of tumor cells. But the gene encoding the estrogen receptor is rarely mutated in breast cancer, which means that it is not a true cancer-causing gene.

Perhaps the most important realization in cancer research since the era when sex hormone receptors were discovered is that particular genes, when they become mutated, can cause a normal cell to turn cancerous. Such genes, once they do mutate, are referred to as oncogenes, and they are believed to be responsible both for initiating the transformation of a normal cell into a cancerous one and for driving tumor growth. That is why breast cancer (like all cancers) is described today as fundamentally a disease of genes. An oncogenic mutation, such as a small change in the DNA nucleotide sequence of a gene, might disable a protective gene or boost the activity of a tumor-promoting one. In some cases, entire genes are deleted or duplicated [*see sidebar on page 93*].

Tumors can now be classified according to the genes that are overactive or suppressed in their cells and according to the resulting changes in the manufacture and function of proteins encoded by those genes. The damaged genes can vary from tumor to tumor, and this heterogeneity at the genetic level explains why breast cancers in individual patients might behave differently. Some cancers have limited invasiveness and metastatic potential, for instance, whereas others spread quickly to distant organs. Knowing the molecular profile of a patient's tumor should permit a doctor to focus on inhibiting the mechanisms driving that particular tumor, one day choosing from an arsenal of drugs a set that will interfere with the specific molecules involved in the initiation, growth and spread of the cancer. The success of trastuzumab and other HER2-targeted therapies illustrates the potential of this approach in combating breast cancer.

Targeting HER2

IN THE EARLY 1980S the gene that gives rise to HER2 was first discovered in mutated form in rat neural tumors by investigators at the Massachusetts Institute of Technology, who named that oncogene *Neu*. Soon researchers realized that the gene was a mammalian version of one previously identified in viruses called *ERBB*, so *Neu* also came to be known as *ERBB2*. This gene was not done accumulating names, however. When scientists identified the protein encoded by *ERBB2*, they realized that it was closely related to a cell-membrane protein called epidermal growth factor receptor (EGFR). Thus, when they finally isolated the human version of the *ERBB2* gene, they named it human epidermal growth factor receptor 2 (*HER2*).

As it turns out, the entire EGFR family of proteins has proved important to tumor cell growth in a variety of cancers. When activated by specific molecules that bind to them (their ligands), such receptors transmit a proliferation signal to the cell by initiating a cascade of internal molecular interactions—spurring activity by genes whose encoded proteins regulate the activity of still more "downstream" genes. Shortly after the *HER2* gene was discovered, scientists noted that it was frequently duplicated in breast cancer cells and that having multiple copies of the gene was associated with a poor prognosis.

Laboratory studies confirmed that adding copies of the *HER2* gene to a normal cell could trans-

THE AUTHORS

FRANCISCO J. ESTEVA is director of the Breast Cancer Translational Research Laboratory at the University of Texas M. D. Anderson Cancer Center, where *GABRIEL N. HORTOBAGYI* is director of the Multidisciplinary Breast Cancer Research Program. Esteva, an associate professor in the departments of breast medical oncology and molecular and cellular oncology, focuses on bridging the gap between basic research and patient treatment. Hortobagyi, a recent president of the American Society of Clinical Oncology, is also chair of, and a professor in, the department of breast medical oncology. His research spans all aspects of breast cancer biology, management and therapeutics and has earned numerous awards.

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form it into a cancer cell—a hallmark ability of oncogenes. Because 20 percent of breast cancer tumors overproduce the HER2 protein, it became a therapeutic target for drug researchers. Genentech scientists created trastuzumab in the late 1980s by manufacturing so-called monoclonal antibodies that bind to the HER2 receptor, preventing it from being activated. In clinical trials, it was found that trastuzumab could lengthen the survival of patients with both early-stage and metastatic breast cancer.

The success of trastuzumab has led to the development of similar antibody-based therapies, such as pertuzumab, which binds to HER2 at a different site than trastuzumab and has the added effect of preventing the receptor from interacting with other members of its family in the cell membrane, such as EGFR and HER3. Blocking such interactions reduces growth signaling along the intracellular pathways of molecular communication downstream of these receptors. Pertuzumab can even disrupt certain types of HER2 activation in tumor cells that have become resistant to trastuzumab. Moreover, we have shown that combining trastuzumab with pertuzumab can boost the rate of cell death in breast cancer cells overproducing HER2.

Still another method of wielding antibodies against the HER2 receptor is to attach a potent toxin to them, which the antibodies then transport into the cancer cell. After the toxin-antibody pair is internalized by a cell, the toxin detaches and kills the cell. This approach has been successful in other types of cancer, such as acute myeloid leukemia, and clinical trials are under way in patients with metastatic breast cancer to determine the safety and efficacy of such trastuzumab-based conjugates.

To send a growth signal into a cell, the intracellular region of the EGFR family of proteins must first be acted on by tyrosine kinases, enzymes that chemically modify a segment known as the tyrosine kinase domain. Tyrosine kinases can thus act as growth-stimulating factors, and inhibiting them directly is another way of squelching EGFR-mediated growth signaling in cells. That is why pharmaceutical companies are avidly pursuing the clinical development of such drugs. Lapatinib (Tykerb) is a dual EGFR/HER2 tyrosine kinase inhibitor that has shown remarkable laboratory results, leading to growth arrest and cell suicide in breast cancer cell lines that overproduce HER2.

One way to improve the effectiveness of HER2-targeted therapy is, therefore, to combine a drug such as trastuzumab with a tyrosine kinase inhibitor such as lapatinib. In breast cancer cell lines, that combination produces greater synergistic growth inhibition and higher rates of cell suicide. Even in cell lines that have developed resistance to trastuzumab after long-term treatment, lapatinib has proved just as effective at inducing cell suicide. A recent large (phase III) clinical trial among patients with HER2-overproducing metastatic breast cancer, whose disease had become resistant to trastuzumab, demonstrated that lapatinib plus capecitabine chemotherapy doubled the median time to progression as compared with capecitabine alone. On the basis of these results, in 2007 the U.S. Food and Drug Administration approved the use of lapatinib combined with

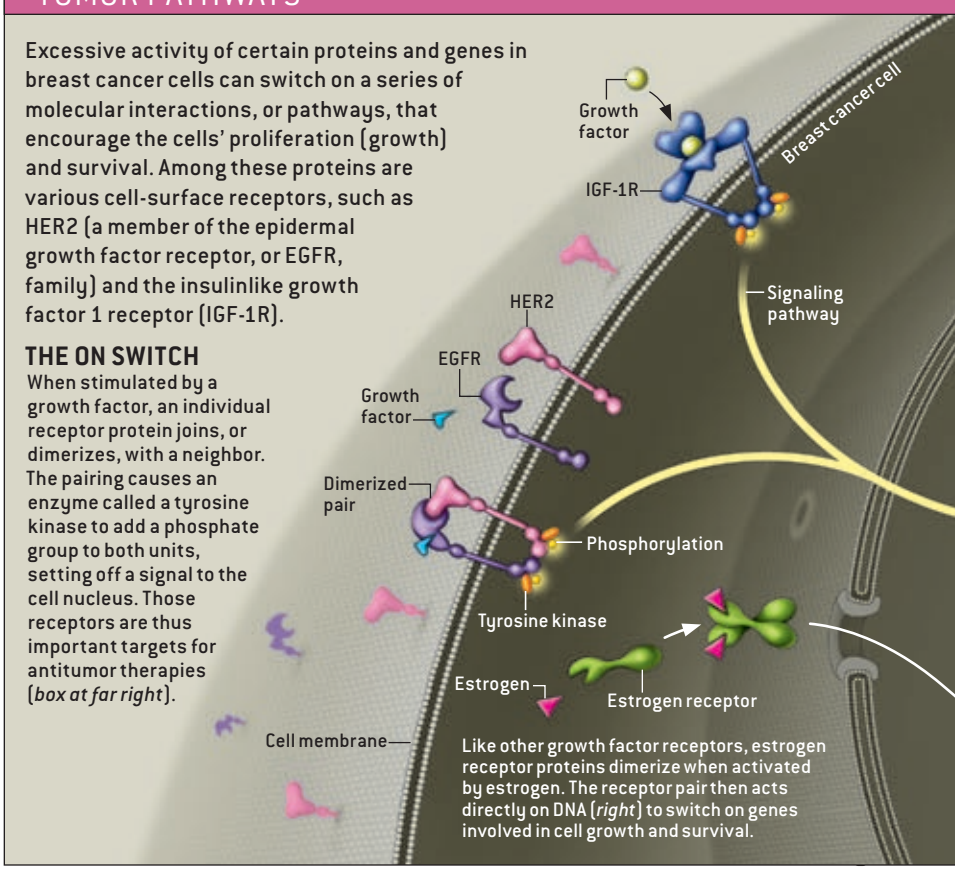
JEN CHRISTIANSEN

TUMOR PATHWAYS

Excessive activity of certain proteins and genes in breast cancer cells can switch on a series of molecular interactions, or pathways, that encourage the cells' proliferation (growth) and survival. Among these proteins are various cell-surface receptors, such as HER2 (a member of the epidermal growth factor receptor, or EGFR, family) and the insulinlike growth factor 1 receptor (IGF-1R).

THE ON SWITCH

When stimulated by a growth factor, an individual receptor protein joins, or dimerizes, with a neighbor. The pairing causes an enzyme called a tyrosine kinase to add a phosphate group to both units, setting off a signal to the cell nucleus. Those receptors are thus important targets for antitumor therapies (*box at far right*).



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capecitabine for treating metastatic disease. Clinical trials to determine lapatinib's value as an adjuvant treatment in a wider variety of circumstances are ongoing, as are trials of several other tyrosine kinase inhibitors that target HER2 and EGFR.

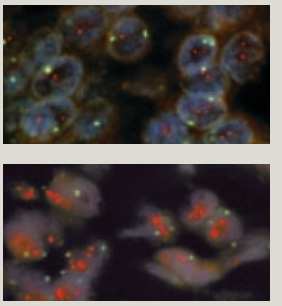
Finding alternative means of interfering with the same growth pathways is important because, as can happen with trastuzumab, cancer cells often do eventually find ways to evade individual drugs. Also under way is research into how and why cancer cells develop resistance to trastuzumab, so that investigators can use those insights as a guide to designing more effective combinations or new agents for patients whose tumors overproduce HER2.

In studies of cell cultures and animals, for instance, our laboratory has discovered that cancer cells employ many different mechanisms to survive in the presence of trastuzumab, including increasing their production of other growth factor receptors, either from the EGFR/HER family or from other families, such as the insulinlike growth factor 1 (IGF-1) receptor. The cells may also lose or inactivate the tumor suppressor gene *PTEN*. This gene normally blocks a survival pathway involving the enzyme phosphotidylinositol 3-kinase (PI3K), which allows damaged cells to ignore signals telling them to commit suicide. We have even seen cells lose or disable the extracellular binding site that trastuzumab attaches to on the HER2 receptor.

In light of these observations, identifying additional molecular targets to attack in cells that overproduce HER2, as well as targets in the other 80 percent of tumors that do not have *HER2* mutations, is a high research priority.

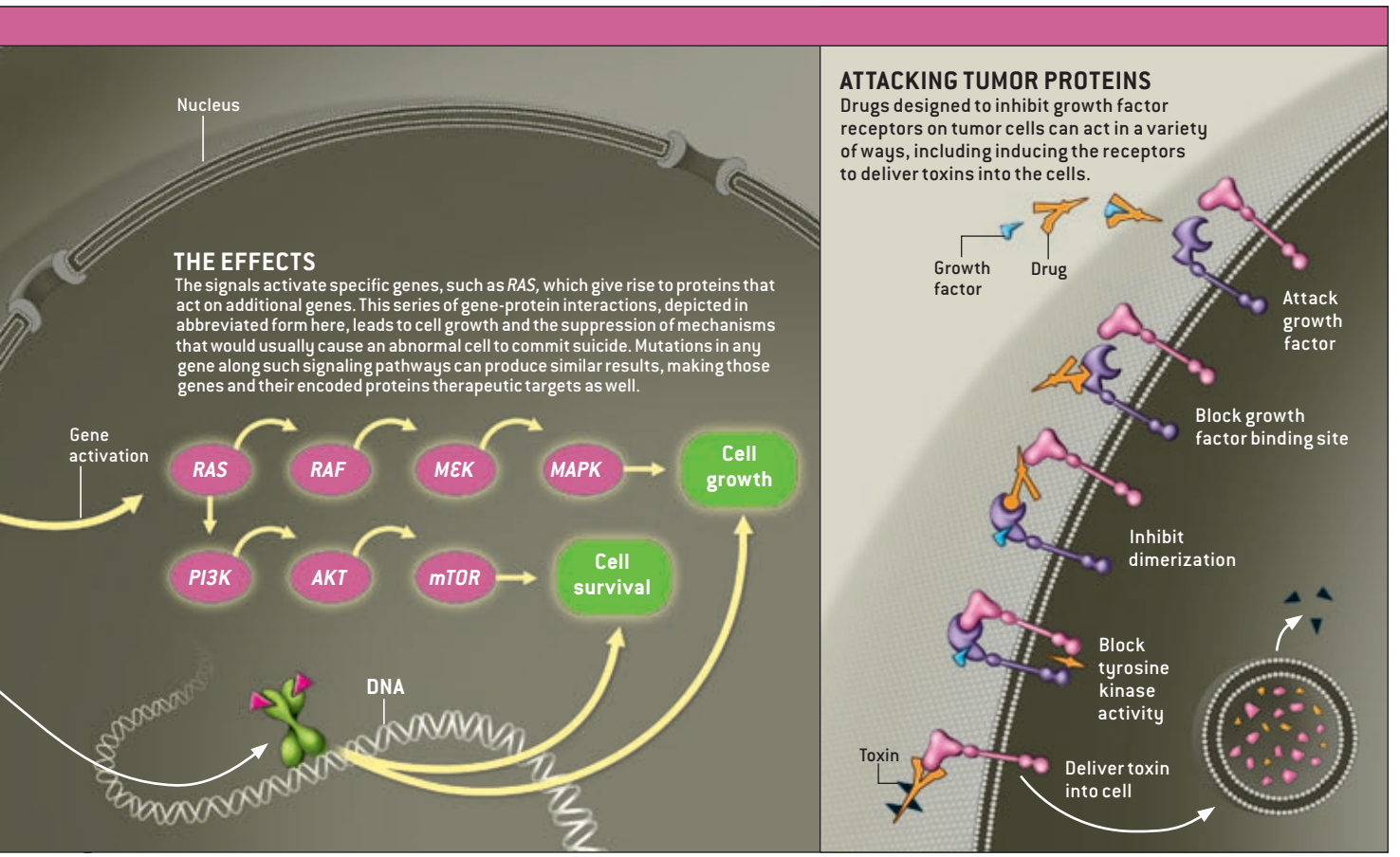
Expanding the Arsenal

AMONG THE MOST promising new targets for breast cancer therapy is the IGF-1 receptor as well as the growth hormone molecules that activate it, IGF-1 and IGF-2. High levels of IGF-1 in the bloodstream have been linked with increased risk of breast cancer, and many laboratory and clinical studies have implicated its receptor in the development, maintenance and progression of multiple cancer types. Signaling by the IGF-1 receptor regulates a variety of cellular processes, including growth, motility and protection from cell suicide. In fact, such signals have been



COURTESY OF FRANCISCO J. ESTEVA

HER2 AMPLIFICATION
A gene encoding the HER2 growth factor receptor is tagged with red fluorescence in breast cancer cells (*top*). In HER2-positive cancer cells (*bottom*), the gene is duplicated many times over, leading to overproduction of HER2 proteins that causes the cells to receive excessive growth signals.


Nucleus
THE EFFECTS
The signals activate specific genes, such as RAS, which give rise to proteins that act on additional genes. This series of gene-protein interactions, depicted in abbreviated form here, leads to cell growth and the suppression of mechanisms that would usually cause an abnormal cell to commit suicide. Mutations in any gene along such signaling pathways can produce similar results, making those genes and their encoded proteins therapeutic targets as well.
Gene activation
RAS
RAF
MEK
MAPK
Cell growth
PI3K
AKT
mTOR
Cell survival
DNA
ATTACKING TUMOR PROTEINS
Drugs designed to inhibit growth factor receptors on tumor cells can act in a variety of ways, including inducing the receptors to deliver toxins into the cells.
Growth factor
Drug
Attack growth factor
Block growth factor binding site
Inhibit dimerization
Block tyrosine kinase activity
Toxin
Deliver toxin into cell

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shown to protect tumor cells from the effects of chemotherapy and radiation therapy. Conversely, inhibiting IGF-1 receptor activity during radiation therapy or chemotherapy has been found to enhance tumor cell suicide rates in animal studies.

In addition to exploring IGF-1 receptor inhibition as a direct therapeutic tool for breast cancer, scientists are evaluating ways to apply it toward preventing or reversing resistance to other treatments, such as endocrine therapies, trastuzumab and lapatinib. Cross talk between the IGF-1 receptor and the various other growth factor receptors—including estrogen, HER2 and additional EGFRs—is a key mechanism for the growth and survival of breast cancer. This codependence and communication between different intracellular pathways is thought to play an important role in drug resistance. Our research group has shown, for example, that blocking the IGF-1 receptor with a monoclonal antibody restores the sensitivity of resistant cells to trastuzumab and disrupts the interaction between the IGF-1 and HER2 receptors. Suppressing the IGF-1 receptor also kills the resistant cells. Furthermore, lapatinib appears to have inhibitory effects on IGF-1 signaling in the trastuzumab-resistant cells, suggesting that its ability to limit tumor cell proliferation may result not only from its anti-EGFR/HER2 activities but also from direct IGF-1 receptor inhibition.

The tangle of signaling pathways leading from the receptors we have been describing to the cellular processes that actually cause a cell to divide or to resist suicide despite DNA damage is highly complex. But scientists are finding that key genes along those pathways are also frequently mutated or dysregulated in tumor cells. Among the best characterized examples is the *PI3K* gene, whose encoded protein chemically modifies another protein known as AKT, which in turn modifies a complex called the mammalian target of rapamycin (mTOR). This PI3K/AKT/mTOR pathway plays a critical role in the body's use of glucose for energy and other important physiological processes in normal cells, but it is pathologically overactivated in cancer cells, prolonging their survival. Because the pathway's effects are ubiquitous in the body, delivering drugs that inhibit it could disrupt healthy cells as well as cancerous ones—a drawback that has so far limited the use of such agents.

Several mTOR inhibitors are nonetheless being tested in clinical trials, both as single agents and combined with other therapies. At the moment, studies using the mTOR-suppressing antibiotic rapamycin, along with an inhibitor of the IGF-1 receptor, suggest that such combinations yield additive antitumor effects as compared with single agents.

Another approach showing great promise is combining direct antitumor agents with compounds that target elements in a tumor's environment. Cancers secrete a variety of growth factors to attract the endothelial cells that build new blood vessels in a process called angiogenesis. Overproduction of the

TARGETED THERAPIES

A growing list of drugs designed to inhibit specific tumor proteins are approved to treat breast cancer patients (*bold*) or are undergoing clinical trials.

DRUG TYPE

- **Aromatase inhibitor** (blocks an enzyme involved in estrogen and progesterone synthesis)
- **Monoclonal antibody** (impedes activation of cellular receptors)
- **Kinase inhibitor** (inhibits signaling by cellular receptors)
- **Vaccine** (stimulates production of antibodies specific to tumor proteins; can be composed of cells or peptide molecules)
- **Other** (includes direct inhibitors of other molecules or gene therapy to alter cellular protein manufacture)

TARGET	DRUG
Estrogen/ progesterone receptor proteins	**Anastrozole** **Letrozole** **Exemestane** **Tamoxifen** **Fulvestrant**
HER2 receptor protein	**Trastuzumab** **Pertuzumab** **Lapatinib** NeuVax dHER2 MVF-HER-2 E1A (gene therapy)
IGF-1 receptor protein	IMC-A12 CP-751, 871 AMG 479 h7C10 OSI-906
PI3K/AKT/mTOR cell survival pathway	BGT226 BEZ235A RAD001 Rapamycin
VEGF receptor protein (involved in forming tumor blood vessels)	**Bevacizumab** Sunitinib Vatalinib Pazopanib AZD2171 AMG706 AMG386 PTC299
Other targets	Dasatinib (SRC inhibitor) THERATOPE Dendritic cell vaccines P53 peptide vaccine ALT801 (p53 inhibitor) Ad5CMV-p53 (gene therapy) Anti-p53 T cell reinfusion AZD2281 (PARP protein inhibitor) BSI-201 (PARP inhibitor)

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Global Power

Targeted therapies will be most powerful, in principle, when they are used together in combinations tailored to the tumor features driving an individual patient's cancer. Clinical trials to test specific drug combinations provide critical information about which treatments work most effectively on different tumor profiles and reveal unexpected interactions between drugs. But trials take time, often years, to enroll a sufficient number of participants to generate statistically significant results. That is why multinational research consortia based in Europe and the U.S. are pooling resources to conduct a 50-country trial, the Adjuvant Lapatinib and/or Trastuzumab Treatment Optimization Study (ALTTO), which has just begun recruiting in the U.S.

Some 1,500 testing sites will treat patients with early (stage I or II) breast cancers that overproduce the HER2 protein, giving them chemotherapy and either trastuzumab or lapatinib alone, or one of those drugs followed by the other, or both drugs together. The trial will provide the first side-by-side comparison of these HER2-targeted treatments that work by different mechanisms.

With a goal of including as many as 8,000 women on six continents, ALTTO has the potential to quickly generate results that can then be applied to patients everywhere. Moreover, this global data-sharing model can highlight differences in treatment responses or toxicity among different ethnic groups, a phenomenon observed with certain types of chemotherapy because of genetic variations that affect the way the drugs are metabolized by patients' bodies. Having such information about the newer targeted therapies will help doctors to further personalize treatment, tailoring it to both the tumor and the patient. ***—The Editors***

most important of these, vascular endothelial growth factor (VEGF), is thought to make tumors more dangerous, and high levels correlate with worse survival rates in human invasive breast cancers. Genentech's bevacizumab (Avastin) is a monoclonal antibody directed against VEGF that was first approved for use in colon cancer in 2004. In more recent clinical trials among patients with heavily treated metastatic breast cancer, bevacizumab alone had limited activity, but certain patients who received it in combination with capecitabine chemotherapy showed improved responses. In another study, HER2-negative metastatic breast cancer progressed more slowly in patients who received paclitaxel chemotherapy with bevacizumab than it did in patients who received paclitaxel alone. Based on such results, bevacizumab was recently approved for use in breast cancer patients, and other VEGF inhibitors are also in development, such as Pfizer's sunitinib (Sutent), a tyrosine kinase inhibitor targeted against the VEGF receptor.

At the same time, very basic biology research is continuing to turn up new molecular targets that both reveal more about the underlying mechanisms of cancer and provide potential leads for drug development. Terumi Kohwi-Shigematsu of Lawrence Berkeley National Laboratory and her colleagues announced one such discovery earlier this year. They identified a gene called *SATB1* as the "master regulator" of activity for more than 1,000 genes involved in breast cancer metastasis. Kohwi-Shigematsu showed that the influence of the SATB1 protein encoded by the gene is both necessary and sufficient for breast cancer cells to become metastatic, which makes it an appealing therapeutic candidate.

Progress in the molecular targeting of breast cancer and individualized therapy will generally rely on the continuing development of profiling tools to determine whether a patient's tumor overproduces proteins such as HER2, SATB1 and others that might be direct drug targets. In addition, genetic testing can help characterize a tumor's overall gene activity patterns—a potential signature of a good or poor prognosis. Still other tests already available or nearing approval can help profile the patient herself to establish whether she has genetic variations that might make her body process a medication more slowly than average—a situation that can be problematic with a drug such as tamoxifen that depends on the body to convert it to active form.

Meanwhile further clinical trials of various drug combinations are needed to validate the effectiveness of multipronged attacks. A 50-country trial has recently begun recruitment in the U.S., for example, to test lapatinib and trastuzumab alone and in combination with each other and with traditional chemotherapies [*see box on this page*].

Such a large trial exemplifies the considerable resources and attention focused on breast cancer research, in recognition of its importance as a global health threat. Doctors' ability to profile a tumor and tailor treatment to fight it with a growing arsenal of weapons is already making a difference in the survival rates of patients, and the coming decade promises even more dramatic progress. SA

MORE TO EXPLORE

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